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Ovine maternal and fetal cardiovascular effects of the serotonergic agonist R(-)-2,5-dimethoxy-4-methyl-amphetamine and characterization of serotonergic receptors in the ovine uterine and umbilical vessels

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by

Lubo Zhang

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GENERAL INTRODUCTION

Explanation of Dissertation Format

This dissertation is written in an alternate thesis format as permitted by the Graduate College. It includes a research objective, a background and literature review, a rationale, an experimental part, a discussion, a summary, a list of references cited in the background and literature review and in the discussion. The experimental part has five sections which correspond to the manuscripts submitted for publication.

This dissertation contains the experimental results obtained by the author during his graduate study under the supervision of his major professor, Dr. Donald C. Dyer. A portion of the results included in Section I were obtained in collaboration with Dr. Miguel Isla.

Research Objective

The purpose of this research was to study the effects of the serotonergic agonists acting on the uterine and umbilical circulation in the near-term pregnant ewe/fetus. Maternal and fetal heart rate and blood pressure, uterine artery blood flow, and fetal umbilical artery blood flow were monitored following maternal administration of the serotonergic agonist and antagonist in the chronically instrumented ewe/fetus model. In addition, isolated uterine artery and umbilical vessels were used to explore in more detail agonist-antagonist receptor mechanisms. The significance of this research is that it documents

under *in vivo* conditions the ability of the serotonergic agonists to alter fetal hemodynamics, their dose-response relationship and, through the use of *in vitro* techniques, the receptor mechanisms involved.

Background and Literature Review

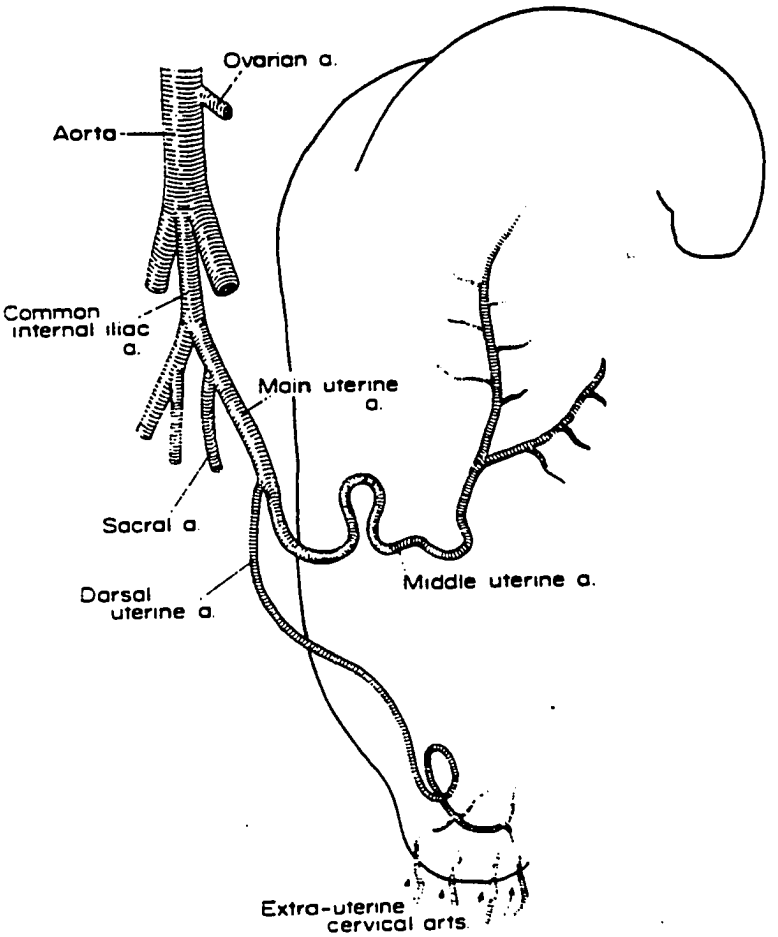
The uterine and umbilical vasculature are the potential sites at which drugs could affect the fetus by producing vasoconstriction. Serotonin causes contraction of most isolated large arteries (Cohen et al., 1981; Cohen et al., 1983; Van Nueten et al., 1982; Van Nueten et al., 1981; Van Nueten et al., 1984) including uterine and umbilical vessels (Altura et al., 1972; Dyer, 1970a; Dyer and Gough, 1971; Nair and Dyer, 1973). In this section the uterine and fetal circulation and serotonin receptors and vascular responses are briefly reviewed.

Uteroplacental and fetal circulation

Uterine circulatory anatomy The uterine artery is a continuation of the umbilical artery which originates from the internal iliac artery. In humans, the abdominal aorta divides into iliac arteries which subdivides into external and internal iliac arteries. In sheep the caudal end of the abdominal aorta trifurcate into two external iliac arteries and a common internal iliac artery. The common internal iliac artery subdivides into two internal iliac arteries that branch into the main uterine and sacral arteries. The main uterine artery is further subdivided into middle and dorsal uterine arteries (Fig. 1).

The uterus receives its blood supply from both the uterine and

Fig. 1. Arterial supply to the ewe uterus (From Fuller et al., 1978)


Ovarian a.
Aorta
Common internal iliac a.
Main uterine a.
Sacral a.
Middle uterine a.
Dorsal uterine a.
Extra-uterine cervical arts.

ovarian arteries. The functional importance of these two vessels varies among different species and within the same species during pregnancy. In guinea pigs, the ovarian artery is the major contributor to the total blood flow of the ovary-oviduct-uterus complex in the nonpregnant state and becomes the lesser contributor at day 11 of pregnancy (Chaichareon et al., 1976). In sheep, the uterus receives its blood supply predominantly from the uterine artery. Using electro-magnetic flow probes and microspheres, Rosenfeld et al. (1974) estimated that in sheep the uterine arteries contributes approximately 80% of the uterine blood flow.

Adjustment of the uterine circulation to pregnancy

Vascular adjustment to pregnancy The diameter of the uterine arteries enlarges several fold during pregnancy. In sheep, the external diameter increases from about 1 mm in the nonpregnant state to 8 or 9 mm in the near-term pregnant state (Assali and Brinkman, 1972). The diameter reverts to the nonpregnant size within one week post partum. The mechanisms by which these reversible changes take place are not quite clear. Moll and Gotz (1985) suggested that the increased diameter of arteries supplying uterine tissue during pregnancy in the guinea pig were due to changes in the nonmuscular elements of the arterial wall and that estrogen may mediate these effects. Guenther et al. (1988) demonstrated that a decrease in the collagen to elastin ratio in the uterine artery in pigs during pregnancy was positively correlated with the observed increase in arterial diameter ($r=.69$, $p<.01$) at physiological pressures. A significant positive correlation between estrogen concentration and uterine arterial diameter suggested that

increasing estrogenic exposure may induce these changes. On the other hand, the marked increase in uterine blood volume and flow during the pregnancy could also play an important role in bringing about the histological changes in the uterine vasculature. The development of the placental circulation which would decrease uterine vascular resistance through the production of estrogen could also contribute to an adjustment in the pattern and size of the uterine vasculature. Whatever the mechanism(s) of the alterations in the structure of the vessels may be, it is clear that the enormous enlargement of the uterine vascular supply is extremely important to the progressively enlarging uterus and developing fetus in order to provide adequate nutrition at all times during pregnancy.

Uterine hemodynamic adjustment to pregnancy Among the various adjustments that take place in the maternal organism to pregnancy two events are considered of paramount importance for both maternal and fetal homeostasis. First is the increase in maternal cardiac output and blood volume (Rosenfeld, 1977). Second is the tremendous rise in uterine blood flow which, near term, reaches 17 to 20 times the nonpregnant values (Assali and Brinkman, 1972). The blood flow to the uterus increases during pregnancy to satisfy the metabolic demands of the conceptus. This is accompanied by an increase in cardiac output in most species. In humans, cardiac output increases approximately 30%-40% in early gestation and then plateaus (Lees et al., 1967). In sheep and goats, the cardiac output increases progressively and reach the maximum in late gestation (Rosenfeld, 1977). Dilts et al. (1969) demonstrated that sheep uterine blood flow increased from about

1.5 ml/min/kg in the nonpregnant state to about 17 ml/min/kg near term pregnancy. The fraction of cardiac output to the uterus increased by about the same magnitude. Using the microsphere technique, Rosenfeld (1977) estimated that the uteroplacental blood flow in sheep received 16% of its cardiac output.

On the other hand, the development of the uteroplacental circulation can be regarded as a low resistance circulatory network which brings about nearly a 10-fold decrease in uterine vascular resistance (Dilts et al., 1969). This decrease in the uterine vascular resistance facilitates the marked increase in uterine blood flow that occurs during pregnancy. The overall systemic vascular resistance of the mother falls by about 30% during pregnancy because of this low uteroplacental vascular resistance (Dilts et al., 1969). The decrease in systemic resistance assists in accommodating the increase in maternal cardiac output during pregnancy.

Regulation of uterine circulation The uterine vascular plexus receives a rich, vasomotor innervation (Bell, 1972) which has been demonstrated to be capable of altering uterine blood flow (Clark et al., 1980). Adrenergic innervation of the uterus is present in every species examined, whereas cholinergic innervation is either scarce or absent (Thorbert et al., 1977). Adrenergic nerves of the uterus originate from peripheral ganglia. In guinea pigs, approximately half of the cervix and a third of the uterine horns receive adrenergic nerve innervation. However, the density of the adrenergic innervation in the uterus progressively decreases during pregnancy (Thorbert et al., 1978). The disappearance of adrenergic nerves starts early in gestation

in those parts of the uterus in close proximity to a fetus and then spreads to the whole organ. It is thought that the response of the uterine adrenergic nerves to pregnancy is part of a regulatory mechanism mediated by steroid hormones. The independent and interdependent actions of both steroid hormones and catecholamine on uterine vascular regulation have been reported (Clark et al., 1978; Ford et al., 1977).

It seems that the uterine vasculature becomes insensitive to neuroadrenergic stimulation as pregnancy progresses and simultaneously becomes hypersensitive to circulatory catecholamine (Ryan et al., 1974). However, no substantial difference between nonpregnant and pregnant sheep in the sensitivity of the uterine blood flow response to neural stimulation and catecholamine injection has been observed (Meschia, 1983). Marked vasoconstriction and reduced uterine blood flow in pregnant sheep has been observed by both nerve stimulation of the uterine artery (Fuller et al., 1979) and infusion of catecholamine into pregnant sheep (Anderson et al., 1977; Rosenfeld et al., 1977). However, it seems that the uterine blood flow is not tonically regulated by the $alpha_1$ receptor since administration of phenoxybenzamine failed to change the uterine blood flow (Chez et al., 1978). During gestation in sheep, the uterine vasculature is more sensitive to catecholamine than are other vascular beds in the systemic circulation and the α_1-adrenoceptor mediated contractile responses to norepinephrine (NE) of uterine arteries increased during pregnancy (Martensson and Carter, 1982).

Placental circulation The placentas of sheep, goats and cattle represent the cotyledonary variety of epitheliochorial placenta. A

crosscurrent arrangement between the maternal and fetal circulation was proposed for the ovine placenta (Meschia, 1983). Data on oxygen transfer indicate that the ovine placenta is an inefficient organ of diffusional exchange (Rankin et al., 1971). In the fetal lamb the umbilical venous Po_2 is consistently lower than the uterine venous Po_2 over a wide range of maternal Po_2 values (Rankin et al., 1971).

In sheep, blood flow to the uterus and its placenta have been measured throughout pregnancy (Rosenfeld et al., 1974). In the early part of gestation, myometrium and endometrium still form the bulk of uterine weight and receive the largest fraction, approximately 73%, of uterine blood flow. Blood flow to the formation sites of the placental cotyledons (caruncles), however, is remarkably high in relation to their size. Consequently, in the 2nd month of ovine pregnancy, placental blood flow becomes the largest fraction (approximately 63%) of the total uterine flow. In the final 2 months of pregnancy fetal weight increases exponentially while at the same time placental weight decreases slightly. Despite the decrease in placental weight, placental blood flow increases several folds from approximately 300 ml/min to as much as 2 liters/min. In the last month of gestation, caruncula flow becomes approximately 82% of uterine flow. It is obvious that any reduction in uterine blood flow will considerably affect placental circulation.

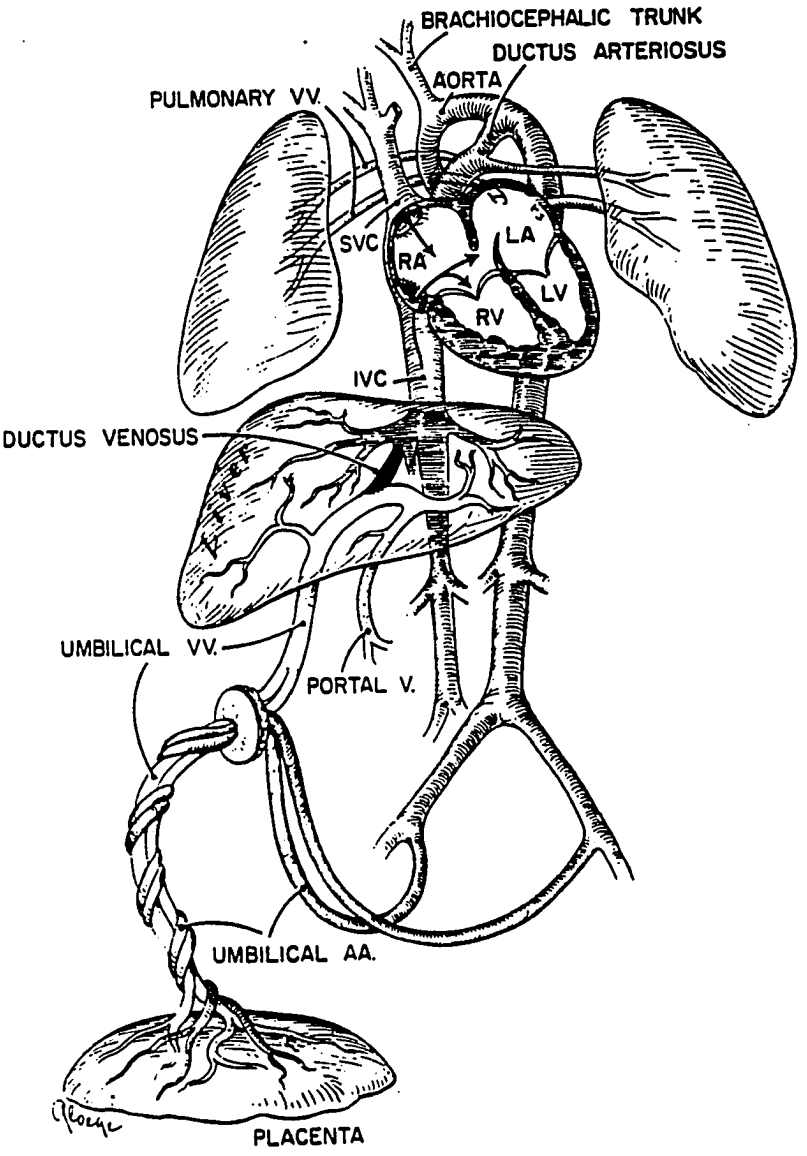
As discussed above, the formation and growth of the placenta vascular bed generates a low vascular resistance in the uterine vessels which brings about a large increase of blood flow to the uterus during pregnancy. Therefore, influencing the development of the placental circulation could modify the magnitude of uterine blood flow. This

would be a chronic regulatory mechanism of the uterine blood flow. On the other hand, rapidly changing the diameter of the placental circulatory channels can act as a short-term regulatory mechanism. It is generally thought that nerves are absent in the umbilical cord and placenta and for this reason attention has been focused on the role of humoral autacoid in the control of placental vascular tone. Most studies have been conducted in vitro because of the technical difficulties of in vivo investigations during pregnancy. In vitro studies have included perfusion of the umbilical vessels (Boura et al., 1979) and recording the vasoconstriction of helical strips of umbilical artery or vein (Dyer, 1970a, 1983; Tulenko, 1979). Mak et al. (1984) demonstrated that thromboxane A_2 in the placenta and thromboxane A_2, 5-HT and bradykinin in the umbilical vein could contribute to control of vascular smooth muscle tone. Their vasoconstrictor effects are partly indirect and affected by the concomitant local release/formation of eicosanoids. These autacoid may normally have important influences on blood flow in the fetal extracorporeal circulation. Epinephrine (EPI), NE and histamine have, if any, minimal influences on the placental circulation.

Fetal circulation

Anatomy of the fetal cardiovascular circulation The fetus takes up nutritional substances and excretes metabolic wastes into the maternal circulation through the placenta. Thus, the placenta serves as the "lung and kidney" for fetus. The major circulatory features of the fetal lamb are diagrammed in Fig. 2. The human fetus differs from the sheep fetus in having one umbilical vein rather than two. The right and

Fig. 2. Schematic representation of cardiovascular circulation of fetal lamb (From Assali et al., 1968)


BRACHIOCEPHALIC TRUNK
DUCTUS ARTERIOSUS
AORTA
PULMONARY VV.
SVC
RA
LA
LV
RV
IVC
DUCTUS VENOSUS
UMBILICAL VV.
PORTAL V.
UMBILICAL AA.
PLACENTA

left ventricles of the fetus operate in parallel because of the foramen ovale between the right and left atria. The patent ductus arteriosus connects the pulmonary artery and the aorta. Therefore, blood is pumped into the aorta at different sites and the lungs are almost entirely bypassed.

The combined output of the left and right ventricle is about equally divided between the placenta and the body of the fetus itself. The placental circulation is supplied by the umbilical arteries and has a relatively low vascular resistance. The rate of blood flow in the descending aorta of the fetus is very high (490 ml/kg fetal weight/min), about half of which goes to the placenta via the umbilical arteries (Rudolph and Heymann, 1974). Most oxygenated blood returning from placental circulation enters the inferior vena cava; about 40% traverses through the foramen ovale to the left heart. Therefore, the left-sided heart chambers are supplied with more highly oxygenated blood from the placenta. Since the lungs are collapsed and the pulmonary vascular resistance is high, about 90% of the output of the right ventricle traverses the patent ductus arteriosus to reach the descending aorta, and only about 7% traverses the lungs in the fetus of the sheep near term (Rudolph and Heymann, 1974).

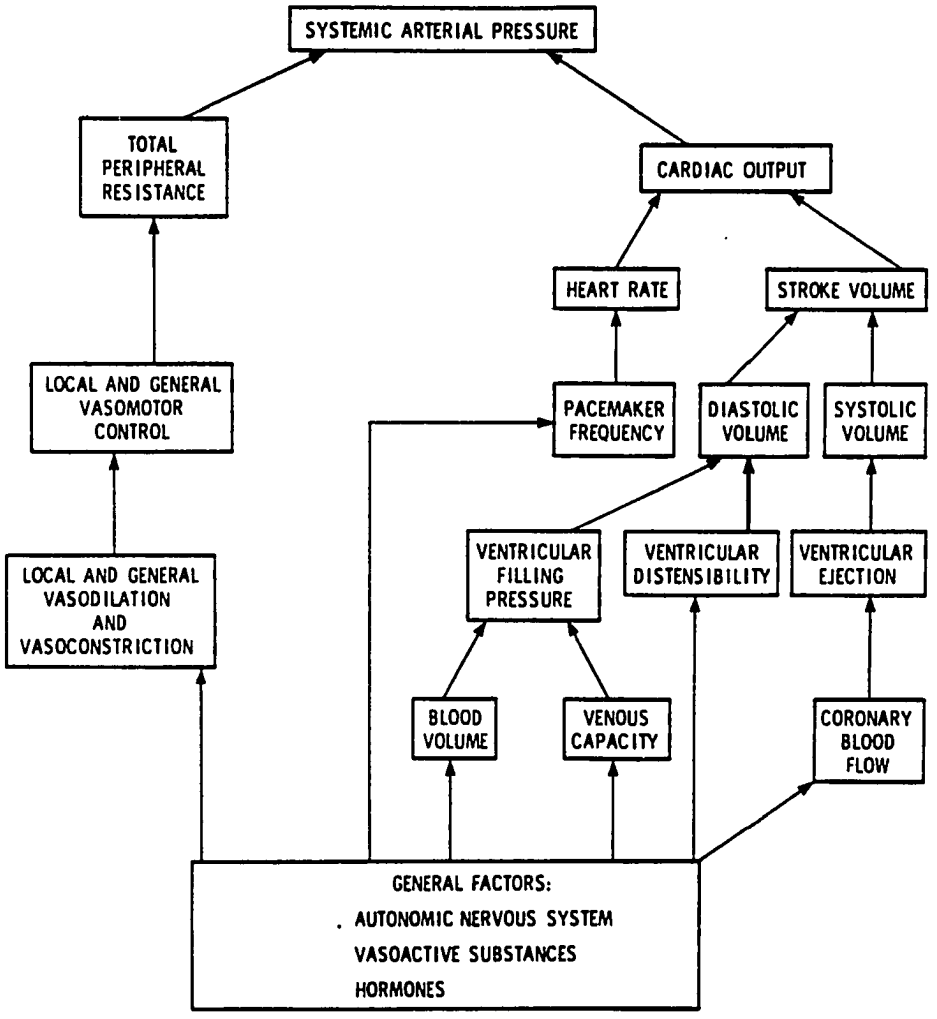
The blood oxygen saturation is about 58% in the umbilical artery and about 80% in the umbilical vein. Part of the oxygenated blood in the umbilical vein bypasses the liver through the ductus venosus and enters the inferior vena cava, where it joins blood from the lower trunk and extremities (26% saturated) and from the liver. Mixed blood reaching the left atrium has an oxygen saturation of 60-65%.

The pulmonary arterial pressure is about 5 mmHg above that in the aorta. Therefore, most of the right ventricular output flow enters the aorta through the ductus arteriosus. The blood enters the aorta at a point distal to the origins of the arteries to the head and upper limbs, and is thus directed to the posterior body and umbilical arteries. More highly oxygenated blood from the left ventricle is pumped into the ascending aorta to reach the arteries supplying the cerebral circulation, the heart, and the upper extremities.

Regulation of the fetal circulation Fig. 3 shows a block diagram of the factors that enter into play to control arterial blood pressure. It has been demonstrated that the fetal heart has limited ability to increase stroke volume (Rudolph and Heymann, 1974) and this limitation could be explained by the lower compliance (Romero et al., 1972) and contractility (Friedman, 1973) of the fetal cardiac muscle. Furthermore, since the two ventricles effectively function in parallel as discussed above, the Frank-Starling mechanism would not be as important as it is in the adult. The fetal cardiac output is thus regulated predominantly by changes in heart rate (Rudolph and Heymann, 1974). In sheep, the fetal heart rate ranges from 170 to 220 and this fast heart rate is important to provide the fetus with the high cardiac output necessary to meet its metabolic activities. In general, sustained bradycardia is thought to reflect fetal distress (Assali et al., 1968).

The fetal systemic arterial pressure is relatively low compared to the adult, which could be caused by the low fetal systemic vascular resistance. The major factor which contributes to the low fetal

Fig. 3. Block diagram of the factors affecting arterial blood pressure (From Assali et al., 1968)


SYSTEMIC ARTERIAL PRESSURE
TOTAL PERIPHERAL RESISTANCE
CARDIAC OUTPUT
HEART RATE
STROKE VOLUME
LOCAL AND GENERAL VASOMOTOR CONTROL
PACEMAKER FREQUENCY
DIASTOLIC VOLUME
SYSTOLIC VOLUME
LOCAL AND GENERAL VASODILATION AND VASOCONSTRICTION
VENTRICULAR FILLING PRESSURE
VENTRICULAR DISTENSIBILITY
VENTRICULAR EJECTION
BLOOD VOLUME
VENOUS CAPACITY
CORONARY BLOOD FLOW
GENERAL FACTORS:
. AUTONOMIC NERVOUS SYSTEM
VASOACTIVE SUBSTANCES
HORMONES

systemic vascular resistance is the umbilicoplacental circulation (Assali et al., 1968). This vascular bed accepts about the half of the fetal cardiac output and has a relatively low vascular resistance. Therefore, any increase in the vascular resistance of the umbilicoplacental circulation promptly increases fetal systemic vascular resistance and produces an increase in arterial pressure and a decrease in cardiac output. It has been demonstrated by Assali et al. (1968) that compression of the whole umbilical cord produces bradycardia along with a transitory hypertension. If the umbilical arteries alone are compressed, similar changes are observed. If the umbilical veins are compressed, a prompt bradycardia with hypotension occurred. These changes are probably vagal in origin and are related to many factors such as hypoxia, decreased right heart filling, cardiac output, and so forth.

The autonomic nervous system supplying the vascular bed is fully developed in the term fetus (Assali et al., 1968). It appears that parasympathetic innervation precedes sympathetic innervation in the fetus (Rudolph and Heymann, 1974). Baroreflex activity has been demonstrated to be present in the fetal lamb by observing a fall in the heart rate following an increase in arterial blood pressure (Maloney et al., 1977; Shinebourne et al., 1972) and its sensitivity progressively increased with advancing gestation until term, but there was no further change after birth (Rudolph and Heymann, 1974).

Sympathetic innervation starts in the fetal lamb at about 0.5 of gestation (Lebowitz et al., 1972). It seems that there is greater sympathetic control of blood pressure in the term fetus and newborn than

in the adult (Woods et al., 1977). From studies of the development of adrenergic nerve control of various organs a general principle has been proposed: the function of the post-synaptic component can generally be demonstrated before the presynaptic elements are fully functional (Rudolph and Heymann, 1974). The studies indicate that alpha and beta adrenergic receptors and parasympathetic receptors are present in the myocardium and many vascular structures at an early gestational age (Rudolph and Heymann, 1974; Vapaavuori et al., 1973).

It has been well established that NE is present in the circulation of near-term fetuses and is derived from the adrenal gland as well as the sympathetic nervous system (Cheung and Brace, 1987; Lewis et al., 1982). Administration of exogenous NE to the fetal lamb produced an increase in arterial pressure and a decrease in heart rate (Cheung and Brace, 1987, 1988; Lewis et al., 1982). In chronically catheterized sheep fetuses, acute hypoxia caused an increase in arterial pressure and a decrease in heart rate (Iwamoto et al., 1983; Lewis et al., 1984). It has been suggested that these cardiovascular changes may be mediated by the concomitant increase in plasma NE and EPI concentrations (Cohen et al., 1984; Jones and Ritchie, 1983; Lewis et al., 1982).

Arginine vasopressin (AVP) may also play an important role in maintaining homeostasis of the fetal cardiovascular system (Stark et al., 1982; Stegner et al., 1984; Wiriyathian et al., 1983). There was a large increase in plasma AVP concentration during fetal stress and acute hypoxia (Stark et al., 1982; Stegner et al., 1984). It has been demonstrated that infusion of AVP produced a marked increase in fetal arterial pressure and a decrease in heart rate (Robillard and Weitzman,

1980; Wiriyathian et al., 1983). The autonomic nervous system may modulate the fetal arterial pressure and heart rate responses to AVP (Tomita et al., 1985).

Serotonin receptors and vascular responses

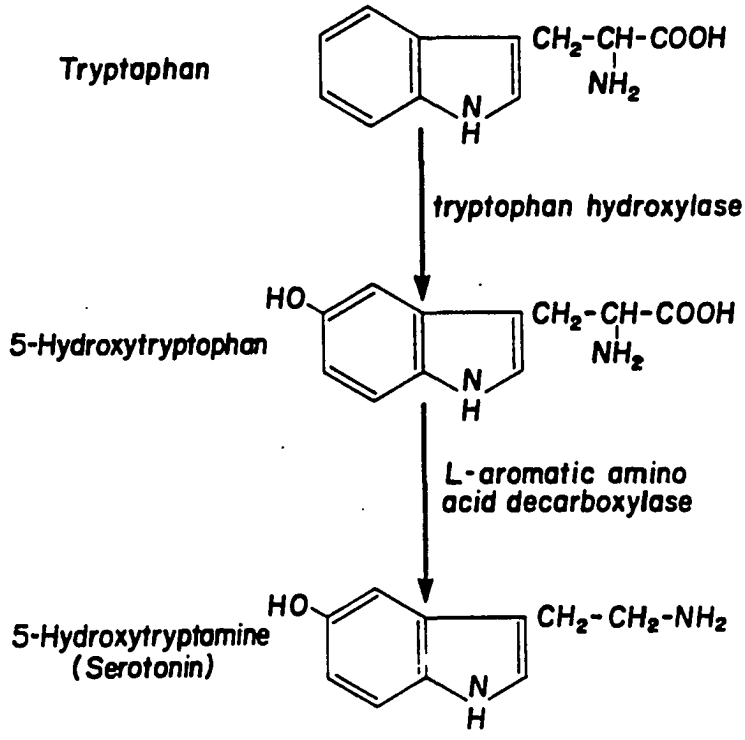
Biochemistry of serotonin

Distribution of serotonin Most of the serotonin in the body is found in enterochromaffin cells of the intestine, the central nervous system, and blood. Platelets contain about 90% of the 5-HT in the blood. The concentration of free 5-HT in the plasma is about 20 ng/ml. The gastrointestinal tract in mice, rats and guinea pigs has been reported to possess serotonergic neurons (Gershon et al., 1977). Other tissues containing significant amounts of 5-HT are the heart, kidney, spleen and thyroid. The significance of the presence of serotonin in these tissues is not yet understood.

Biosynthesis of serotonin Serotonin is synthesized from the essential amino acid L-tryptophan by two enzymatic reactions (Fig. 4). The amount of 5-HT synthesized daily is about 10 mg. The rate-limiting step in the synthesis of serotonin from tryptophan is the first reaction catalyzed by tryptophan hydroxylase, which is present only in cells synthesizing serotonin. The second enzyme, L-aromatic amino acid decarboxylase, is widely distributed in tissues and is not specific. It will catalyze the decarboxylation of all aromatic amino acids, including L-3,4-dihydroxyphenylalanine, the precursor of the catecholamine.

Metabolism of serotonin The major pathway of metabolism of serotonin is by oxidative deamination with the production of the unstable intermediate 5-hydroxyindoleacetaldehyde, which can undergo

Fig. 4. Biosynthesis of serotonin (From Tyce, 1985)

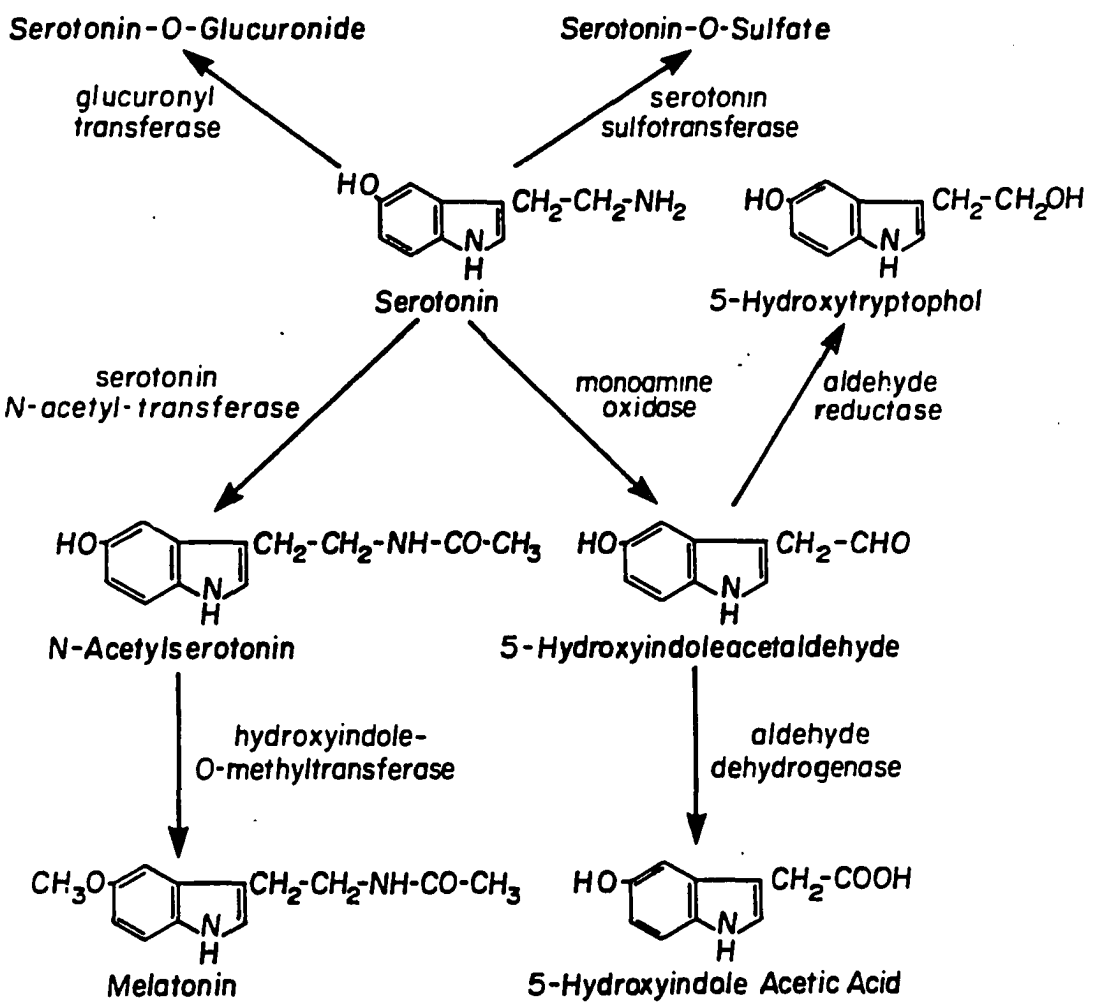

Tryptophan
CH2-CH-COOH
NH2
N
H
tryptophan hydroxylase
HO
5-Hydroxytryptophan
CH2-CH-COOH
NH2
N
H
L-aromatic amino acid decarboxylase
HO
5-Hydroxytryptamine (Serotonin)
CH2-CH2-NH2
N
H

oxidation catalyzed by the enzyme aldehyde dehydrogenase with the formation of 5-hydroxyindoleacetic acid, or undergo reduction catalyzed by aldehyde reductase with the formation of 5-hydroxytryptophol (Fig. 5). Under normal circumstances the predominant pathway is oxidation to 5-hydroxyindoleacetic acid.

Classification of serotonin receptors

Overview The first classification of 5-HT receptors came from the work of Gaddum and Picarelli (1957) who demonstrated the existence of two pharmacologically distinct types of receptors (D and M) for 5-HT in the guinea-pig ileum. The D receptor is sensitive to Dibenzyline (phenoxybenzamine) and mediates contraction of the smooth muscle. The M receptor is sensitive to morphine and mediates depolarization of the cholinergic nerves. Since Dibenzyline and morphine are not specific 5-HT receptor blocking drugs, they are of little or no value as tools for the overall classification of 5-HT receptors. Recently, two classification schemes of 5-HT receptors have been proposed (Bradley et al., 1986; Peroutka and Snyder, 1979). Based on radioligand binding study, Peroutka and Snyder (1979) demonstrated that there were two distinct 5-HT binding sites (5-HT_1 and 5-HT_2) in the central nervous system (CNS), while Bradley et al. (1986), based largely on data from the functional studies in isolated peripheral tissues, proposed three distinct groups of 5-HT receptors which have been named "5-HT_1-like", 5-HT_2 and 5-HT_3. 5-HT_1 binding sites in CNS the have been further subdivided into four subtypes (Heuring and Peroutka, 1987; Pazos et al., 1984b; Pedigo et al., 1981). More recently, Hartig (1989) proposed a classification scheme for 5-HT receptors based primarily on

Fig. 5. Metabolism of serotonin (From Tyce, 1985)


Serotonin-O-Glucuronide
Serotonin-O-Sulfate
glucuronyl transferase
serotonin sulfotransferase
HO
CH2-CH2-NH2
N
H
Serotonin
HO
CH2-CH2OH
N
H
5-Hydroxytryptophol
serotonin N-acetyl-transferase
monoamine oxidase
aldehyde reductase
HO
CH2-CH2-NH-CO-CH3
N
H
N-Acetylserotonin
HO
CH2-CHO
N
H
5-Hydroxyindoleacetaldehyde
hydroxyindole-O-methyltransferase
aldehyde dehydrogenase
CH3O
CH2-CH2-NH-CO-CH3
N
H
Melatonin
HO
CH2-COOH
N
H
5-Hydroxyindole Acetic Acid

the molecular structure of the receptor proteins and second messenger systems.

5-HT_1 receptors 5-HT_1 binding sites first proposed by Peroutka and Snyder (1979) were defined by nanomolar affinity for 5-HT and could be labeled with [^{3}H]5-HT. Later it has been found that 5-HT_1 binding sites labeled by [^{3}H]5-HT were heterogeneous (Table 1). The different affinities to spiperone at [^{3}H]5-HT binding sites led to the suggestion that the sites with high affinity for spiperone should be designated 5-HT_{1A} sites, while the sites with relatively low affinity for spiperone should be designated 5-HT_{1B} sites (Pedigo et al., 1981; Schnellmann, 1984). A third subtype of 5-HT_1 recognition sites (the 5-HT_{1C} sites) was identified in the choroid plexus and cortex of various species (Pazos et al., 1984a, 1984b; Peroutka, 1986). Most recently, the studies of the pharmacology and biochemistry of the 5-HT_{1C} receptor (Hartig, 1989; Pazos and Palacios, 1985; Yagaloff and Hartig, 1985) suggest that it should be more appropriately placed in the 5-HT_2 family of receptors.

The 5-HT_{1A} receptor was the second 5-HT receptor that was successfully cloned. The transfection of the G-21 clone into monkey kidney cells produces a protein that exhibits the ligand binding characteristics of the 5-HT_{1A} and beta-adrenergic receptors (Fargin et al., 1988). Both ^{125}I-labeled cyanopindolol, a radioligand that labels beta-adrenergic receptors, and the 5-HT_{1A} agonist [^{3}H]8-hydroxy-(2-di-N-propylamino)-tetralin (8-OH-DPAT) have a high affinity to G-21-transfected monkey kidney cell membranes. The possible relationship between 5-HT_{1A} receptors and β-adrenergic receptors are supported by the

Table 1. Characteristics of 5-HT receptor subtypes (From Schmidt and Peroutka, 1989)

	5-HT_1 receptors		
	5-HT_{1A}	5-HT_{1B}	5-HT_{1D}
Molecular biology	Cloned	?	?
Radiolabeled by	[^{3}H]5-HT [^{3}H]8-OH-DPAT [^{3}H]Ipsapirone [^{3}H]WB 4101 [^{3}H]Buspirone [^{3}H]PAPP [^{3}H]Spiroxatrine [^{3}H]5-MeO-DPAC ^{125}I-labeled BH-8-MeO-N-PAT	[^{3}H]5-HT ^{125}I-labeled CYP (Rat and mouse only)	[^{3}H]5-HT
High density regions	Raphe nuclei Hippocampus	Substantia nigra Globus pallidus	Basal ganglia
	Selective		
Potent pharmacologic agents (<10nM)	8-OH-DPAT Ipsapirone	No agents available	No agents available
	Nonselective		
	5-HT 5-CT RU 24969 d-LSD (-)Pindolol	5-HT 5-CT RU 24969 (-)Pindolol	5-HT 5-CT Metergoline
Second messenger	Inhibition of adenylate cyclase	Inhibition of adenylate cyclase	Inhibition of adenylate cyclase
Membrane effects	Hyperpolarization via an opening of potassium channels	?	?
Other functional correlates	Basilar artery Thermoregulation Hypotension Sexual behavior 5-HT Syndrome	Autoreceptor	Autoreceptor

Table 1. continued

5-HT$_2$ receptors			5-HT$_3$ receptors
5-HT$_{1C}$	5-HT$_{2A}$	5-HT$_{2B}$	5-HT$_3$
Cloned	?	Cloned	?
[^{3}H]5-HT [^{3}H]Mesulergine [^{3}H]Mianserin ^{125}I-labeled LSD [^{3}H]SCH 23390 ^{125}I-labeled SCH 23892	[^{3}H]DOB [^{77}Br]R(-)DOB ^{125}I-labeled DOI [^{3}H]Ketanserin	[^{3}H]Spiperone [^{3}H]Mesulergine ^{125}I-labeled LSD [^{3}H]Ketanserin [^{3}H]Mianserin ^{125}I-labeled methyl-LSD [^{3}H]N-methyl-spiperone	[^{3}H]GR 65630 [^{3}H]ICS 205-930 [^{3}H]Quipazine [^{3}H]Zacopride [^{3}H]BRL 43694 [^{3}H]QICS 205-930
Choroid plexus	Layer IV cortex	Layer IV cortex	Peripheral neuron Entorhinal cortex Area postrema
		Selective	
No agents available	DOB DOI	No agents available	GR 65630 Zacopride ICS 205-930 Granisetron Ondansetron
		Nonselective	
Mesulergine Metergoline Methysergide Mianserin	Mesulergine Metergoline Ketanserin Spiperone	Mesulergine Metergoline Methysergide Mianserin Ketanserin Spiperone	
PI turnover	?	PI turnover	?
Depolarization via an opening of chloride channels	?	Depolarization	Depolarization
?		Vascular contraction Platelet shape change Platelet aggregation Paw edema Tryptamine seizures Head twitches	Transmitter release von Bezold-Jarisch reflex

similarity in amino acid sequence shared by these two receptors. In several amino acid stretches, the 5-HT_{1A} receptor more closely resembles the β_2-adrenoceptor than it does the other cloned 5-HT receptors. The 5-HT_{1A} receptor and the β_2-adrenoceptor are identical in 20 out of 27 amino acids, while the 5-HT_{1A} receptor only shares a maximum of ten out of 27 amino acids with either of the other two cloned 5-HT receptors (Fargin et al., 1988; Julius et al., 1988; Pritchett et al., 1988). This raises intriguing questions about the functional and evolutionary relationships among these different receptors.

5-HT_{1A} receptors can be labeled by a number of radioligands. Although the 5-HT_{1A} site can be labeled with [^{3}H]5-HT, it can be more directly labeled with [^{3}H]8-OH-DPAT (Gozlan et al., 1983; Hall et al., 1985; Hoyer et al., 1985; Peroutka, 1986), [^{3}H]ipsapirone (Dompert et al., 1985), [^{3}H]buspirone (Moon and Taylor, 1985) and [^{3}H]1-(2-(4-aminophenyl)ethyl)-4-(3-trifluro-methylphenyl)piperazine (PAPP) (Asarch et al., 1985). A selective α_1-adrenergic radioligand, [^{3}H]WB 4101, has also been demonstrated to label the 5-HT_{1A} site (Norman et al., 1985). 8-OH-DPAT potently binds at the 5-HT_{1a} site (IC_{50} = 6.3 nM) but is essentially inactive (IC_{50} = 3800 nM) at both the 5-HT_{1B} and 5-HT_2 binding sites (Arvidsson et al., 1981; Gozlan et al., 1983; Marcinkiewicz et al., 1984).

The dense areas for 5-HT_{1A} binding sites in the brain are the CA 1 region, the denate gyrus of the hippocampus and the raphe nuclei (Hoyer et al., 1986; Pazos and Palacios, 1985). The involvement of 5-HT_{1A} receptor in 5-HT behavioral syndrome (Smith and Peroutka, 1986; Tricklebank, 1985), seminal emissions and/or ejaculations (Kwong et al.,

1986) and the thermoregulation (Tricklebank, 1985) has been proposed. In the periphery, 5-HT-induced contractions of the canine basilar artery have been proposed to be a functional correlate of the 5-HT_{1A} receptor (Peroutka et al., 1986; Taylor et al., 1986).

^{125}I-labeled cyanopindolal directly labeled 5-HT_{1B} binding sites in the rat brain (Hoyer et al., 1985). Other radioligands for 5-HT_{1B} binding sites have also been reported (Table 1). Three arylpiperazines related compounds, m-trifluoromethyl-phenylpiperazine (TFMPP), 1-(3-chlorophenyl)piperazine (mCPP) and 1-(2-methoxyphenyl)piperazine (2-MPP) have been identified as relatively selective ligands for the 5-HT_{1B} binding sites (Fuller et al., 1980; Glennon, 1987; Sills et al., 1984). The affinities of these three compounds were much higher at 5-HT_{1B} binding sites than they were at 5-HT_{1A} and 5-HT_{1C} binding sites. TFMPP has K_i values of 1950 and 30 nM for the 5-HT_{1A} and 5-HT_{1B} binding sites, respectively (Glennon, 1987). mCPP has K_i values of 2400 and 75 nM for the 5-HT_{1A} and 5-HT_{1B} binding sites, respectively (Glennon, 1987). 2-MPP possesses a 100-fold selectivity for 5-HT_1 vs. 5-HT_2 sites and an affinity for 5-HT_1 sites comparable to that of TFMPP (Glennon, 1987).

The 5-HT_{1B} receptor binding sites appear to be species specific. To date, the 5-HT_{1B} binding sites have been identified only in rat and mouse brains but not in guinea pig, cow, chicken, turtle, frog, or human brain membranes (Heuring et al., 1986; Hoyer et al., 1986). In the rat brain, the highest density of 5-HT_{1B} binding is in globus pallidus, dorsal subiculum, and substantia nigra (Hoyer et al., 1986). The 5-HT_{1B} site has been associated with 5-HT and noradrenergic neurons "autoreceptors" in the CNS and periphery (Engel et al., 1986; Raiteri et

al., 1986). No functional correlates of the 5-HT_{1B} receptor in blood vessels have been reported.

The 5-HT_{1D} site was originally identified in bovine brain membranes (Heuring and Peroutka, 1987) and found in many other species, including pig, calf, and human brain membranes (Schmidt and Peroutka, 1989). These [^{3}H]5-HT labelled sites display nanomolar affinity for 5-carboxyamidotryptamine (5-CT), 5-methoxytryptamine, metergoline, and 5-HT but display a low affinity to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_2 and 5-HT_3 selective agents (Heuring and Peroutka, 1987). 5-HT_{1D} binding sites exist in all regions of bovine brain but are most dense in the basal ganglia (Heuring and Peroutka, 1987). To date, a specific physiological effect of 5-HT has not been correlated with 5-HT_{1D} binding sites.

As discussed above, the 5-HT_1 radioligand binding site is clearly not a homogeneous entity. Since no selective antagonists have yet been described, it is a considerable handicap to the definition of these sites related to functional studies. In this sense, Bradley et al. suggested a term "5-HT_1-like" receptors which may not necessarily be identical to 5-HT_1 recognition sites as described above. Some currently used 5-HT_1 antagonists like methiothepin and methysergide bind to both 5-HT_1 and 5-HT_2 receptors (Bradley et al., 1986; Engel et al., 1983; Martin and Sanders-Bush, 1982). The criteria for a response to be defined as mediated through "5-HT_1-like" receptors have been proposed (Bradley et al., 1986) in which the response should be susceptible to antagonism by methiothepin and/or methysergide and should be resistant to antagonism by selective 5-HT_2 receptor antagonists such as ketanserin and cyproheptadine and by 5-HT_3 receptor antagonists such as MDL 72222

or ICS 205-930. Also the response should be mimicked by the selective 5-HT_1 agonist 5-CT at concentrations equal to or less than those of 5-HT.

According to these criteria, 5-HT-induced contractile responses in the saphenous vein of the dog and the basilar artery from rabbit and man have been defined as "5-HT_1-like" receptor mediated (Apperley et al., 1986; Bradley et al., 1986a, 1986b; Feniuk et al., 1985; Forster and Whalley, 1982; Peroutka, 1984). There is also evidence that such receptors mediate vasoconstriction in the arteriovenous anastomoses located in the carotid circulation in vivo (Verdouw et al., 1985). The 5-HT-induced relaxation of some vascular and gastrointestinal smooth muscle could also be mediated by "5-HT_1-like" receptors (Cohen et al., 1983; Feniuk et al., 1983, 1984).

5-HT_2 receptors 5-HT_2 receptors have first been proposed by Peroutka and Snyder (1979) to describe a 5-HT recognition site in the brain with a low affinity for 5-HT but with a high affinity for spiperone and other 5-HT receptor antagonists such as methysergide and cyproheptadine. Other radioligands that have been used to label 5-HT_2 sites include [^{3}H]ketanserin, [^{3}H]mesulergine, [^{125}I]LSD, and [^{125}I]MIL (Hoffman et al., 1985; Leysen et al., 1982; Yagaloff and Hartig, 1985). Ketanserin, a 5-HT antagonist, possesses a high affinity (K_i < 1 nM) for 5-HT_2 sites and a negligible affinity for 5-HT_1 recognition sites (Arvidsson et al., 1981; Leysen et al., 1981; Leysen et al., 1982). In general, 5-HT_2 binding sites have high affinity for the agents that are usually considered as being serotonin antagonists. On the other hand, classical 5-HT agonists generally display a relatively low affinity for

5-HT_2 sites. One of the good examples is 5-HT. The affinity (K_i) of 5-HT for [^{3}H] ketanserin-labeled 5-HT_2 sites is in the 400-1000 nM range (Glennon, 1987). The highest level of 5-HT_2 binding sites is in layer IV of the cerebral cortex and caudate. Other brain regions have substantially fewer binding sites (Hoyer et al., 1986; Pazos and Palacios, 1985).

It was thought that 5-HT_2 sites were homogeneous (Peroutka, 1988). However, recent studies have provided data to suggest that subtypes exist within the 5-HT_2 receptor family (Lyon et al., 1987; Peroutka et al., 1988; Pierce and Peroutka, 1989). It has been demonstrated that 5-HT competition curves with [^{3}H]ketanserin in rat and human cortical membranes are shallow (Hill slopes = 0.67, 0.69, respectively), suggesting that [^{3}H] ketanserin labels more than one population of 5-HT receptors, while in bovine cortical membranes, 5-HT competition curves are steep (Hill slope = 0.97) indicating that a single site is labeled by [^{3}H] ketanserin (Pierce and Peroutka, 1989). In addition, it was shown that [^{3}H]DOB (4-bromo-2,5-dimethoxy-amphetamine) does not label an agonist state of the 5-HT_2 recognition site, but rather a distinct subtype of the 5-HT_2 receptor (Lyon et al., 1987). Also [^{77}Br] R(-)DOB-labeled sites comprise only 19% of the density of 5-HT_2 sites in rat cortex and are not eliminated by the addition of 10^{-4}M GTP (Peroutka et al., 1989). In accordance with these data it has been proposed that the high affinity component of [^{3}H]ketanserin binding site was designated the 5-HT_{2A} binding site, while the low affinity component was designated the 5-HT_{2B} site (Pierce and Peroutka, 1989) (Table 1). Further elaboration on the classification of the 5-HT_2 receptors will

depend on the development of selective agents that can distinguish subtypes of the 5-HT_2 receptors.

Based on the similarities in amino acid structure and the second messenger pathway, the 5-HT_{1C} receptor has recently been proposed to belong to the 5-HT_2 receptor family (Hartig, 1989; Hoyer, 1988; Pierce and Peroutka, 1989; Pritchett et al., 1989). Similarities in ligand binding properties and second messenger coupling of the 5-HT_2 and 5-HT_{1C} receptors have been apparent (Hoyer, 1988). The high homology between the 5-HT_2 and 5-HT_{1C} receptor clones is obvious and they share 78% sequence homology with 141 out of 180 amino acids being identical (Fargin et al., 1988; Julius et al., 1988; Pritchett et al., 1988).

The identification of ketanserin as a 5-HT_2 antagonist provided a particularly useful tool with which to characterize these receptors pharmacologically. This compound shows negligible affinity for 5-HT_1 binding sites and is not a potent calcium antagonist (Leysen et al., 1981; Van Nueten and Vanhoutte, 1981). However, it should be noted that ketanserin does show appreciable affinity for α-adrenoceptors (Kalkman et al., 1982; Leysen et al., 1981; Leysen et al., 1982; Van Nueten et al., 1981; Wenting et al., 1984) and it is imperative to dissociate those effects of 5-HT which are mediated via stimulation of specific 5-HT receptors from those effects which may be mediated via α-adrenoceptors (Feniuk, 1984). Therefore, before the receptor subserving a given response can be characterized as being of the 5-HT_2 type, it should be necessary to exclude the possible involvement of alpha-adrenergic receptor activity in 5-HT-induced responses or to determine the antagonistic potencies of both ketanserin and methysergide which has

appreciable affinity for 5-HT_1 receptors but has negligible affinity for α-adrenoceptors. The criteria for the characterization of 5-HT_2 receptors have been proposed by Bradley et al. (1986) in which the 5-HT-induced response should be susceptible to antagonism by ketanserin and/or methysergide but not by MDL 72222 and ICS 205-930.

The characterization of 5-HT_2 receptors with the corresponding pharmacological responses has been investigated in various tissues, including blood vessels. Vasoconstriction in many arteries has been found to be mediated by a receptor similar to the 5-HT_2 binding site (Leysen et al., 1984; Maayani et al., 1984; Van Nueten et al., 1981, 1984). Similarly, drug antagonism of tracheal smooth muscle contraction, in vivo bronchoconstriction and contraction of guinea pig ileum is consistent with mediation by 5-HT_2 receptors (Leysen et al., 1984). 5-HT induction of platelet shape changes and aggregation may also be mediated by 5-HT_2 receptors (Leysen et al., 1984). A number of CNS effects of 5-HT have also been attributed to 5-HT_2 receptors (Glennon et al., 1983; Leysen et al., 1982, 1984; Peroutka et al., 1981).

5-HT_3 receptors The recognition of 5-HT_3 receptors is based on the identification of the selective 5-HT_3 receptor antagonists cocaine and MDL 72222 (Fozard et al., 1979; Fozard, 1984a). With the availability of these specific antagonists, 5-HT_3 receptors have been identified in a variety of locations on peripheral efferent and afferent neurons (Fozard, 1984b). Lately, a more potent 5-HT_3 antagonist ICS 205-930 and a selective 5-HT_3 agonist, 2-methyl-5HT, were identified by Richardson et al. (1985). The criteria for the characterization of 5-

HT_3 receptors have been proposed (Bradley et al., 1986a). For a response to be defined as being mediated by 5-HT_3 receptors it should be susceptible to antagonism by cocaine, MDL 72222 or ICS 205-930, but resistant to antagonism by ketanserin or methiothepin. Also this response should be mimicked by the selective 5-HT_3 agonist 2-methyl-5-HT, which has a potency similar to that of 5-HT itself.

Although 5-HT_3 receptors were originally demonstrated on peripheral neurons where they were responsible for eliciting the depolarizing action of 5-HT, recent data show that 5-HT_3 binding sites are also identified in the rat brain with [^{3}H]GR 65630 and a variety of other radioligands (Kilpatrick et al., 1987) (Table 1). 5-HT_3 agonists such as 5-HT, 2-methyl-5-HT, and phenylbiguanide have a moderate affinity (K_i value approximately 150 nM) for this site. The 5-HT_3 antagonists such as granisetron, ICS 205-930, and zacopride display subnanomolar affinity (K_i values = 0.1-0.8 nM) for this site (Schmidt and Peroutka, 1989). In rat brains, 5-HT_3 binding sites are most dense in cortical areas and the area postrema. The possibility that 5-HT_3 receptors may also be found in non-neuronal locations should not be excluded.

It also appears that heterogeneity may exist within the 5-HT_3 class of receptors. Both MDL 72222 and ICS 205-930, which are potent and selective 5-HT_3 antagonists, have been demonstrated to possess significantly different potencies in various physiological systems (Donatsch et al., 1984; Richardson et al., 1985). Three subtypes of the 5-HT_3 receptors have been suggested (Richardson et al., 1985), but more rigorous studies with selective antagonists are needed for their proper characterization.

Second messenger systems linked to 5-HT receptors Most recently it has been suggested that neurotransmitter receptors fall into two broad superfamilies: the G protein receptor superfamily and the ligand-gate ion channel superfamily (Lester, 1988). 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D} and 5-HT_2 receptors are coupled to G proteins and belong to the G protein receptor superfamily (Hartig, 1989). The 5-HT_3 receptors are unique among 5-HT receptors because activation of 5-HT_3 receptors invariably results in a "nicotine-like" fast depolarization (Fozard, 1984a; Mawe et al., 1986). Therefore, it is conceivable that these sites may be very tightly coupled to ion channels and indeed these channels may be part of the receptor as is the case for the nicotine receptor (Neijt et al., 1988). In this sense, 5-HT_3 receptors appear to belong to the ligand-gate ion channel superfamily (Hartig, 1989).

The 5-HT_1 receptor family has been linked to the modulation of adenylate cyclase. 5-HT_{1A} receptors are indisputably linked to adenylate cyclase in brain tissue although whether activation (via stimulatory guanosine triphosphate binding protein, G_s) or inhibition (via inhibitory guanosine triphosphate binding protein, G_i) of the enzyme occurs depends on the pre-existing state of activation (Fozard, 1987). Both activation and inhibition of adenylate cyclase activity by the 5-HT_{1A} receptor has been shown in the 5-HT_{1A} receptor clone transfected functional response system (De Vivo and Maayani, 1986; Markstein et al., 1986; Shenker et al., 1985; Weiss et al., 1986). In the rat hippocampus, 5-HT_{1A} sites are also coupled to neuronal K^+ channels by a pertussis toxin-sensitive G protein which is neither G_s nor G_i (Andrade et al., 1987). In contrast, 5-HT_{1B} sites appear to be

linked in a negative way to adenylate cyclase in rat substantia nigra (Bouhelal et al., 1988; Neale et al., 1987). 5-HT_{1D} receptor also appears to mediate the inhibition of adenylate cyclase activity (Schoeffter et al., 1988).

The 5-HT_{1C} receptor, unlike other 5-HT_1 receptors, is linked to the phosphatidylinositol (PI) turnover. The 5-HT_{1C} receptors are densely present in choroid plexus in brain. It has been demonstrated that the midpoints for the binding and phosphoinositide activation by 5-HT are nearly identical in this area and the response is inhibited by mianserin, ketanserin and spiperone (Conn et al., 1986; Sanders-Bush and Conn, 1987). These data suggest that 5-HT_{1C} receptors in choroid plexus mediate 5-HT-induced PI turnover and there is a single ligand binding site on this single-subunit receptor (Conn et al., 1986; Sanders-Bush and Conn, 1987).

It has been well demonstrated that 5-HT_2 receptors are coupled to PI hydrolysis in the CNS (Berridge et al., 1982; Brown et al., 1984; Conn and Sanders-Bush, 1984, 1987; Kendall and Nahorski, 1985; Pierce and Peroutka, 1988) and in peripheral tissues (Coughlin et al., 1984; de Coureelles et al., 1985; Doyle et al., 1986; Roth et al., 1984). Stimulation of 5-HT_2 receptors leads to activation of phospholipase C. The breakdown of phosphoinositides generates inositol 1,4,5-triphosphate (IP_3), which is known to mobilize calcium from intra-cellular stores and 1,2-diacylglycerol, which activates the Ca^{2+}-sensitive, phospholipid-dependent protein kinase C. On the other hand, extracellular calcium has also been proposed to play an important role in the vascular contractions produced by 5-HT_2 receptor activation (Berta et al., 1986;

Capponi et al., 1987; Nakaki et al., 1985). It has been shown that 5-HT stimulated phosphatidylinositol turnover in rat aorta and that in this tissue the mechanism of the 5-HT-induced contraction required the participation of the opening of a voltage-gate calcium channel and the stimulation of a phosphatidylinositol-specific phospholipase C (Nakaki et al., 1985; Roth et al., 1986). It has been reported that 5-HT stimulated depolarization of the smooth muscle membranes (Fujiwara et al., 1982), which could open the voltage-dependent calcium channel and induce an influx of extracellular calcium. Recent evidence indicates that a rise in cytosolic calcium also activates the phospholipase C (Eberhard and Holz, 1987; Fisher and Agranoff, 1981; Kendall and Nahorski, 1984, 1985). Small increases in cytosolic Ca^{2+} induced by Ca^{2+} influx across the plasma membrane may result in higher cytosolic Ca^{2+} concentration due to activation of phospholipase C which in turn increases IP_3 turnover and release of Ca^{2+} from intracellular stores. The IP_3 produced in this way could also act in the plasma membrane to increase Ca^{2+} influx as it does on the membrane of the responsive fraction of endoplasmic reticulum (Kuno and Gardner, 1987; Slack et al., 1986). The role of IP_3 in eliciting Ca^{2+} entry by emptying an intracellular Ca^{2+} pool is also proposed (Putney et al., 1989). Diacylglycerol is also generated by the activation of phospholipase C following Ca^{2+} influx, which will stimulate protein kinase C activation.

The extracellular calcium can traverse the cell membrane and gain access to the contractile apparatus through two distinct pathways. One is the receptor-operated calcium channel system; the other is the voltage-dependent calcium channel system (Bolton, 1979; Meisheri et al.,

1981; VanBreeman et al., 1982). The voltage-dependent calcium channel is voltage sensitive and as membrane potential is made increasingly more positive the inward calcium current will rise progressively until it reaches a peak magnitude (Brown et al., 1981). A number of calcium entry blocking agents can affect voltage-dependent calcium channels. These compounds have been divided into three groups (Spedding, 1985). Group I consists of nifedipine and related 1,4-dihydropyridines. Group II contains the calcium blockers verapamil, D600, diltiazem and diclofurime which, for the most part, have unrelated chemical structures. Group III is made up of diphenylalkylamine compounds such as cinnarizine, fendiline, flunarizine and prenylamine. The compounds in groups I and II are potent and selective on calcium channels in cardiac muscle. The compounds in group III appear to be less selective and can act on both calcium and sodium channels in cardiac muscle. It has been shown, moreover, that verapamil, D600, and diltazem, namely group II compounds, have approximately equiactive effects on calcium channels in cardiac muscle and vascular smooth muscle. However, nifedipine and related dihydropyridines exert preferential effects on vascular smooth muscle (Janis and Triggle, 1983). It has been known for many years that many isolated smooth muscle preparations can be stimulated to contract by exposing them to a membrane-depolarizing high potassium bathing medium. These potassium-induced tension changes are usually quite sensitive to the inhibitory action of calcium entry clocking agents (Godfraind, 1983; Hurwitz et al., 1980). Contractions produced by high concentrations of potassium can be blocked by adding a calcium entry blocking agent to the high potassium bathing solution or

aborted by adding such an agent after the contraction has been induced (Godfraind, 1983; Hurwitz et al., 1982). Potassium induced contraction can also be abolished by removing calcium ions from the high potassium bathing solution and reinstated by adding calcium ions back to the solution (Hurwitz et al., 1980). These findings indicate that an essential step in the development of a potassium-induced contraction is the influx of calcium ions from the extracellular environment.

Although receptor-operated calcium channels play an important role in the functional behavior of smooth muscle, they have received much less study than have votage-dependent calcium channels. Meisheri et al. (1981) demonstrated in the rabbit aorta that D600 preferentially inhibited potassium induced calcium influx, whereas amrinone preferentially inhibited NE induced calcium influx. They also observed that the calcium fluxes stimulated by the high potassium medium and by norepinephrine were additive. Stice et al. (1987) also demonstrated in pig uterine arteries that D600 or amrinone selectively inhibited ^{45}Ca uptake and contraction induced by either high-K^+ or phenylephrine. Moreover, the blockade of Ca^{2+} uptake by D600 and amrinone was additive. In sum, these experimental results favor the proposal that receptor-operated calcium channels constitute one population and that voltage-dependent calcium channels constitute another population of membrane calcium channels. Whether or not membrane potential exerts any essential or modulating influence on the activation of receptor-operated calcium channels is still unresolved.

5-HT and cardiovascular responses

Cardiovascular effects of central serotonergic neuronal activation It has been demonstrated that activation of an ascending serotonergic system by stimulation of the midbrain raphe produces an increase in blood pressure which is mediated in part by the hypothalamus and which requires transmission toward the spinal cord to enhance sympathetic activity (Robinson, 1982). Administration of serotonin into the rat lateral or the third ventricle also produces an increase in arterial blood pressure and this pressor response can be antagonized by intraventricular administration of the serotonergic antagonists 2-bromolysergic acid diethylamide (BOL) and methysergide (Kristic and Djurkovic, 1980; Lambert et al., 1978). The pressor response to intraventricular administration of serotonin is antagonized by the systemic administration of α-adrenergic antagonists such as phenoxybenzamine and tolazoline, suggesting that the pressor response produced by serotonin probably involves the discharge of the sympathetic nervous system (Kristic and Djurkovic, 1980).

Serotonin and vascular responses Serotonin has complex and multiple actions on cardiovascular function. In an intact animal, it causes either increases or decreases in blood pressure, depending on the species studied and the dose used. The local effect of 5-HT on blood flow can be affected by many factors. Depending on the species, the vascular bed, the experimental conditions, the degree of sympathetic tone, and the route of administration, serotonin can augment or reduce blood flow. It has been demonstrated that 5-HT can produce constriction or dilation of blood vessels (Vanhoutte, 1982a, 1982b; Van Nueten, 1983;

Van Nueten and Vanhoutte, 1984).

Serotonin causes contraction of many isolated large arteries, which can be inhibited by ketanserin, spiperone, methysergide, and a number of other serotonergic antagonists (Cohen et al., 1981; Cohen et al., 1983; Van Nueten et al., 1982; Van Nueten et al., 1981; Van Nueten et al., 1984). There is a good correlation between the affinity of these serotonergic antagonists for 5-HT_2 binding sites in brain tissue and their potency to antagonize contractions of isolated blood vessels to 5-HT, suggesting that vasoconstrictions produced by 5-HT are mediated by 5-HT_2 receptors on the vascular smooth muscle (Leysen et al., 1982; Van Nueten et al., 1981; Van Nueten et al., 1984). However, there are large differences in sensitivity to 5-HT in different blood vessels. Serotonin is more potent than norepinephrine in coronary, cerebral, umbilical, and pulmonary arteries (Cohen et al., 1983; Dyer, 1970a; Edvinsson et al., 1983; Van Nueten, 1983). Vasoconstrictions produced by 5-HT in cutaneous and subcutaneous arteries are inhibited by α-adrenergic antagonists, indicating that activation of α-adrenergic receptors is involved in the responses produced by 5-HT in these vessels (Apperley et al., 1976; Maggi et al., 1983; Winkelmann et al., 1976). In certain pathological states in animals and in humans the vasoconstriction responses in some vascular beds become hypersensitive to serotonin (Hollenberg, 1988; Winquist and Bohr, 1983).

Many large veins constrict when exposed to 5-HT, which is mediated by 5-HT_2 receptors. In the rat jugular vein the response to 5-HT is inhibited competitively by ketanserin, spiperone, and methysergide (Cohen et al., 1981). However, in canine cutaneous veins the

contractions produced by 5-HT are mediated by α-adrenergic receptors (Clement et al., 1969; Curro et al., 1978).

It has been proposed that the contractions evoked by serotonin can be attributed to the activation of 5-HT_2 receptors on the smooth muscle or to the amplification of the contractile response to other vasoconstrictor neurohumoral mediators (Vanhoutte, 1985). In some vessels, direct activation of alpha-adrenoceptors or displacement of other endogenous vasoconstrictors, mainly NE from adrenergic nerves can also be involved in the response produced by 5-HT (Vanhoutte, 1985).

Although 5-HT produces a vasoconstriction effect on most vascular smooth muscle, it can cause relaxation in coronary arteries, cerebral arteries, saphenous veins, pulmonary vein, the canine nasal circulation, and the perfused vascular bed of the guinea pig stomach (Cohen et al., 1983; Edvinsson et al., 1983; Eyre, 1975; Feniuk et al., 1983; Fu and Toda, 1983; Van Nueten et al., 1981; Zhang and Dyer, unpublished data). Activation of β-adrenergic receptors may be involved in the relaxation produced by 5-HT in cerebral arteries (Edvinsson et al., 1983). However, in many other blood vessels in which 5-HT produces relaxation, serotonergic receptors appear to be involved. These receptors differ from 5-HT_2 receptors of the vascular smooth muscle which mediate the contractions. Indeed, the relaxation of many vascular smooth muscles obtained in vitro with 5-HT can be inhibited by methiothepin, methysergide, pizotifen, and cyproheptadine, but not by ketanserin (Cohen et al., 1983; Feniuk et al., 1983; Van Nueten et al., 1981; Van Nueten et al., 1984). Serotonergic receptors mediating relaxation could be located on the vascular smooth muscle cells themselves or the

endothelial cells on vascular smooth muscle which could release endothelium dependent relaxing factor (EDRA) upon the activation of serotonergic receptors (Vanhoutte, 1985). 5-HT can also activate the prejunctional 5-HT_1 receptors with a resulting reduction of the evoked release of NE or cause release of inhibitory transmitters from peptidergic nerves (Vanhoutte, 1985).

5-HT may play a role in regulation of the heart rate. Indeed, the monoamine produces a positive chronotropic effect in atria of the kitten which is not reversed by ketanserin (Kaumann, 1983). The receptor involved is different from 5-HT_1 and 5-HT_2 binding sites. 5-HT also has a positive inotropic effect on the mammalian heart (Buccino et al., 1967). One of the mechanisms could be that 5-HT stimulates the serotonergic receptors on nerve terminals and triggers the release of the adrenergic neurotransmitter (Thandroyen et al., 1985). It has also been reported that 5-HT can inhibit the reuptake of NE by sympathetic nerve terminals (Thandroyen et al., 1985). However, 5-HT does not regulate cardiac contractility under basal conditions (Sole et al., 1979).

5-HT during pregnancy 5-HT has been implicated in a number of physiological and pathophysiological conditions associated with pregnancy and uterine hemodynamics. These include pre-eclampsia, abortion, and parturition. It has been demonstrated that 5-HT concentration increased in whole blood during normal pregnancy (Carter et al., 1982; Carl, 1985; Krupp and Krupp, 1960). Because the fetal content of 5-HT is high, the excess 5-HT might originate in the fetus (Jones and Rowesel, 1973). In rats and mice, placental content of

serotonin has been found to increase throughout gestation (Robson and Senior, 1964). Placental serotonin falls sharply just prior to the time of parturition in the rat, but not in mice. In the rat, administration of a monoamine oxidase inhibitor increased placental serotonin and caused a delay in parturition but this action was not observed in mice (Lesinski et al., 1967). The source of serotonin is unknown. It seems unlikely that 5-HT originates from mast cells since the rat placenta does not contain mast cells. The biosynthetic pathway for serotonin also has been described and approximately 20 ng of serotonin can be formed from 5-hydroxytryptophan per gram of human placenta per hour (Klinge et al., 1964).

Direct investigations into the effect of 5-HT on the uterine vasculature have been extremely limited. 5-HT has been observed to produce death of fetal mice when it was given to the mother. 5-HT produces such an action presumably by reducing the blood supply to the placenta (Robson and Sullivan, 1966). Dyer and Gough (1971) demonstrated that 5-HT and LSD potently constrict human uterine arteries obtained from non-pregnant uteri. With main uterine arterial blood flow monitored via electromagnetic flow transducers, Clark et al. (1980) demonstrated in chronically catheterized ewes that 5-HT produced dose related decreases in uterine blood flow in both pregnant and non-pregnant ewes. The vasoconstrictor responses to 5-HT were significantly attenuated by the 5-HT antagonist, methylsergide. In contrast, responses to NE were not modified by methylsergide, suggesting that specific 5-HT receptors existed and mediated the vasoconstriction produced by 5-HT in the ovine uterine vasculature.

It has been well documented that 5-HT produces a potent vasoconstriction on the umbilical vasculature in humans and in animals (Dyer, 1970a; Bjoro and Stray-Pederson, 1986; Mak et al., 1984; McGrath et al., 1985; Tuncer et al., 1985). These responses are inhibited by cinanserin, bromlysergic acid diethylamide (BOL), ketanserin, mianserin, and methysergide (Dyer, 1974, 1983; McGrath et al., 1985; Tuncer et al., 1985), suggesting that 5-HT_2 receptors are involved in 5-HT-induced vasoconstriction. Contractions to 5-HT can be potentiated 8-10 fold by cocaine and the magnitude of the potentiation was linearly related to an inhibition of 5-HT uptake by the ovine umbilical artery (Dyer, 1970b). Contractions to 5-HT were not associated with changes in either cAMP or cGMP levels (Fiscus and Dyer, 1981). Indomethacin effectively blocked contractions induced by arachidonic acid and adenosine triphosphate (ATP) in sheep umbilical vasculature but did not antagonize contractions to 5-HT, suggesting that release of prostaglandins is not involved in the response to 5-HT but may be for ATP (Fiscus and Dyer, 1982). Sheep umbilical veins and arteries contain 0.38 and 0.13 μg/g wet weight of 5-HT, respectively (Dyer and Weber, 1971).

Involvement of 5-HT receptors in the action of 2,5-dimethoxy-4-methyl-amphetamine Certain phenalkylamine hallucinogens possess a high affinity for 5-HT receptors in the isolated rat fundus preparation (Glennon et al., 1980; Glennon and Mack, 1978). There also appears to be a correlation between 5-HT receptor affinity and hallucinogenic potency in these hallucinogens. The more potent agents usually possess a higher affinity for 5-HT receptors (Glennon et al., 1980; Glennon et al., 1982). 2,5-Dimethoxy-4-methyl-amphetamine (DOM) is a prototypic

phenalkylamine hallucinogen and has been shown to produce a vasopressor action in humans (Snyder et al., 1967), cats (Tadepalli et al., 1975), rats (Huang and Ho, 1972) and dogs (Cheng et al., 1973) which was antagonized by serotonergic antagonists. Dyer et al. (1973) found that DOM produced a potent vasoconstriction on isolated sheep umbilical blood vessels. The vasoconstriction to DOM is effectively antagonized by the serotonergic antagonists cinanserin and BOL (Dyer, 1974, 1983).

Recent evidence suggests that DOM acts via 5-HT_2 receptors (Glennon et al., 1984; Titeler et al., 1985). Both ketanserin and pirenperone are effective in attenuating the discriminative stimulus effects of DOM (Glennon et al., 1983). In radioligand binding studies, an excellent correlation was found between the affinities of hallucinogens at the 5-HT_2 binding site and their potencies to substitute for DOM as a discriminative cue (Glennon et al., 1984). DOM displays a relatively low affinity for 5-HT_1 sites (K_i = 3550 nM) but high affinity for 5-HT_2 binding sites (K_i = 60 nM) (Glennon, 1987). More recently, Sanders-Bush et al. (1988) demonstrated that DOM stimulated phosphoinositide hydrolysis in the cerebral cortex with a maximum effect that is 76% of that produced by 5-HT. This effect of DOM was blocked by the 5-HT_2 antagonists ketanserin and spiperone, but not by antagonists of other phosphoinositide hydrolysis-linked receptors (α_1 adrenergic, histaminergic and muscarinic cholinergic). Taken together, these studies suggest that DOM might produce its hallucinogenic effects via a direct agonistic interaction at 5-HT_2 sites.

Rationale

Serotonergic agonists potentially are harmful to the fetus in that they activate serotonergic receptors and produce vasoconstriction in the umbilical vasculature. Another potential site at which these drugs could affect the fetus is via constriction of the uterine vasculature during pregnancy. 5-HT can constrict human uterine vascular smooth muscle obtained from nonpregnant uteri. However, information is lacking on the effects of 5-HT agonists on uterine arteries from pregnant animals. While most studies have been carried out on isolated tissues, it is important to study the effects of serotonergic agonists on uterine and umbilical artery blood flow hemodynamics in the living animal. The establishment of the chronically instrumented pregnant ewe/fetus model in Dr. Dyer's laboratory allows us to ascertain whether selected serotonergic agonists will alter the maternal and fetal heart rate and arterial blood pressure, the maternal uterine artery blood flow and the fetal umbilical artery blood flow when they are administrated to the mother. Vascular resistances of the uterine and umbilical arteries can then be ascertained, which permits the determination of whether serotonergic agonists exert a vasoconstrictor effect on these vessels. By monitoring fetal arterial blood Po_2, Pco_2 and pH values, it can be determined whether the serotonergic agonists cause stress to the fetus.

The use of isolated uterine and umbilical vascular smooth muscle in association with modern pharmacological receptor analysis techniques permits an in-depth analysis of receptor mechanisms involved in the vasoconstriction produced by the agonists. The application of specific

vasoconstriction produced by the agonists. The application of specific serotonergic agonists and antagonists in the methodology and the determination of the dissociation constants for the agonists and antagonists permit the generation of fundamental information which is needed for classification of serotonergic receptors. These data will also provide the information necessary for a proper comparison of serotonergic receptors reported in other tissues. Fundamental information on the signal transduction system coupling to serotonergic receptors can be obtained in part by using $^{45}Ca^{2+}$ uptake techniques.

The methodology and techniques described above permit correlation of *in vivo* and *in vitro* studies, which allows an understanding of the effects of serotonergic agonists in the pregnant animal and the receptor mechanisms involved.

SECTION I. CARDIOVASCULAR ACTION OF R(-) 2,5-DIMETHOXY-4-METHYL-AMPHETAMINE (DOM) IN THE PREGNANT EWE AND FETAL LAMB[1]

Abstract

Pregnant ewes and their fetuses were instrumented between 110 to 120 days of gestation (term, 145 days) for monitoring heart rate, blood pressure, uterine artery blood flow, and fetal intra-abdominal umbilical artery blood flow and arterial blood gases. DOM (2,5-dimethoxy-4-methyl-amphetamine) was administered intravenously to the ewe in doses of 1, 2.5, 5, 10, or 20 μg/kg. Maternal administration of DOM produced dose-dependent increases in maternal blood pressure and a fall in the heart rate. Uterine blood flow was dramatically decreased by DOM and uterine vascular resistance increased by 6.8, 16.7 and 19.6 fold after DOM at 5, 10, and 20 μg/kg, respectively. These responses were accompanied by fetal hypoxemia, combined metabolic and respiratory acidosis, hypertension and bradycardia. The changes in blood flow and vascular resistance were always smaller in the fetal umbilical artery than those in the maternal uterine artery. The maximal rise in vascular resistance in the fetal umbilical artery was 2.6 fold after DOM (20 μg/kg). Ketanserin (1 mg/kg), administrated 30 minutes prior to the administration of DOM, inhibited maternal and fetal cardiovascular and blood gas responses to DOM. The conclusions are: (1) the fetus is distressed following maternal administration of DOM brought about by a

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reduction in the uterine blood flow and impairment of oxygen transfer to the fetus, (2) the cardiovascular responses to DOM may be mediated by 5-HT_2 receptors.

Introduction

2,5-Dimethoxy-4-methyl-amphetamine (DOM) has a hallucinogenic effect in humans. It is about 50-100 times more potent than mescaline, but is 30-50 times less active than lysergic acid diethylamide (LSD) (Snyder et al., 1967; Hollister et al., 1969). The hallucinogens related to DOM have a peripheral action on the cardiovascular system that must be considered when they are studied. In human subjects DOM (5 mg) elicited an increase in systolic blood pressure and pulse rate (Snyder et al., 1967). The vasopressor action of DOM has also been shown in cats, rats and dogs and was antagonized by serotonergic antagonists (Tadepalli et al., 1975; Huang and Ho, 1972; Cheng et al., 1973). DOM enhanced vasopressor responses to tyramine, norepinephrine (NE) and epinephrine (EPI) but reduced the pressor responses to bilateral carotid artery occlusion (Cheng et al., 1973). Recent evidence suggests that DOM can act on 5-HT_2 receptors (Dyer, 1983; Glennon et al., 1984; Titeler et al., 1985).

We have shown that DOM potently constricts sheep uterine and umbilical blood vessels <u>in vitro</u> (Zhang and Dyer, 1989a, 1989b). Contractions to DOM were effectively antagonized by the ketanserin, a specific 5-HT_2 antagonist (Leysen et al., 1981; Leysen et al., 1982; Van Nueten et al., 1981). The pA_2 values for ketanserin vs. DOM in the

uterine artery and umbilical artery were 8.33 and 9.10, respectively (Zhang and Dyer, 1989a, 1989b). During pregnancy, uterine blood vessels supplying oxygen and nutrients to the developing fetus are maximally dilated, but readily constricted in the presence of vasoconstrictors (Meschia, 1983). Another potential site at which drugs could affect the fetus is via constriction of the umbilical vasculature. In general, drugs which have good lipid solubility cross the placenta with ease. A positive correlation has been established for psychotomimetic potency and increased lipid solubility (Nichols et al., 1977).

To date, there are no published reports of the effects of DOM on fetal cardiovascular function and oxygenation. The purpose of this study was to investigate the cardiovascular and blood gas responses in the pregnant ewe and her fetus to maternal intravenous administration of DOM. Maternal uterine artery blood flow and fetal intra-abdominal umbilical artery blood flow were continuously monitored with the ultrasonic pulsed Doppler flow transducers. Additionally, ketanserin was administrated to the ewe prior to DOM injection to determine the inhibitory effect of ketanserin on the maternal and fetal cardiovascular and blood gas responses to DOM.

Methods

Abbreviations

MBP	maternal arterial blood pressure
FBP	fetal arterial blood pressure
MHR	maternal heart rate

FHR fetal heart rate

MUABF maternal uterine artery blood flow

FUmABF fetal intra-abdominal umbilical artery blood flow

PCVR percent change in vascular resistance

Animal preparation and surgical procedures

Mixed-breed ewes were obtained from a local source and bred by natural mating. The gestational age was based on the day of mating. Robillard et al. (1979) found a good correlation between fetal weight and gestational age using this technique (fetal body weight (Kg) = [0.096 x gestational age (days)] -9.2228). Pregnant ewes of known gestational age (110-120 days) were instrumented during surgery as described below.

Prior to surgery the animals were deprived of food for 48h and water for 24h. General anaesthesia was induced by intravenous injection of thiopental sodium (20mg/kg). The ewe was incubated and maintained on a halothane/oxygen mix (1-2% halothane, approx. 3L/min). The animal was then placed on her right lateral recumbancy. Dorsally at the level of the tuber coxae, a vertical incision (approx. 25 cm) was made about 3 cm cranial to the tuber coxae on the left flank. The left main uterine artery was located in the broad ligament of the uterus. A length of the vessel (approx. 2 cm) was cleared by blunt dissection and a Doppler flow transducer was placed around the vessel. Lidocaine was applied to the blood vessels to help prevent contraction brought on by manipulation. The transducer leads were then sutured to the broad ligament to minimize twisting. Thereafter, the uterine horn was exposed and, through a small uterine incision, the fetal hind limbs were located. The upper part of

the fetus was kept inside the uterus. During fetal surgery, lidocaine was applied to the blood vessels as described above. Polyvinyl catheters (I.D., 0.66 mm; O.D., 1.3 mm) were inserted into the fetal femoral artery and vein for a distance of approximately 5 cm and secured with silk ligatures. Through a midline abdominal incision, using blunt dissection as much as possible, both left and right intra-abdominal umbilical arteries were exposed. Doppler flow transducers were then attached to both vessels. After the incision was closed, the leads of the Doppler flow transducers were secured with silk sutures and anchored to the skin. A catheter was secured to the abdominal skin for access to the amniotic cavity for measuring intrauterine fluid pressure and administration of antibiotics to the fetus. After securing all the fetal catheters and transducers, the uterine incision was closed in two layers. Thereafter, the ewe abdominal wall was closed in separate layers and all catheters and leads of Doppler flow transducers were exteriorized through a subcutaneous tunnel and placed in a cloth pouch on the ewe's flank, after which the skin was closed. For maternal instrumentation, an incision (approx. 4 cm) was made parallel to the tracheal on the left side of the neck. The left carotid artery and jugular vein (approx. 2 cm) were cleared by blunt dissection. Another incision (approx. 2 cm) was made in the middle of the two shoulders and a stainless steel tube was pushed underneath the skin from the incision on the shoulders to the one on the neck. Two tygon catheters (I.D., 1mm; O.D. 1.8 mm) for the carotid artery and jugular vein were introduced through the stainless steel tube from the side of the shoulders to that of the neck. The stainless steel tube was then

removed from the side of the neck. The ends of the catheters on the side of the shoulders, which were connected with a piece (approx. 4 cm) of thick wall tygon tube (I.D., 0.8 mm), were sutured to the skin. The tygon tubes were plugged by a blunt 18 gauge needle with one end sealed. The other ends of the catheters in the side of the neck were ready for inserting into the vessels. For catheterizing the carotid artery, a small hole was made in the vessel by puncture using an 18 gauge needle. The catheter was introduced through the hole into the carotid artery and advanced to the ascending aorta for blood pressure monitoring and arterial blood sampling. The catheter was fixed to the carotid artery by making a purse suture around the catheter. Similarly, a catheter was introduced into the jugular vein and passed to the superior vena cava. This catheter was used for drug administration. The neck incision was then closed. Ampicillin 2 g was given to each ewe i.m. just prior to the surgery and injected into the amniotic cavity at the end of surgery.

Before using, all catheters were soaked in 7% TDMAC heparin complex (Polysciences, Inc., Warrington, PA) to prevent blood clot formation. The catheters were then sterilized with ethyleneoxide. Following surgery, all catheters were filled with heparinized saline solution (20 units heparin/ml). Ewes were housed in individual pens and provided with water and a standard diet. The animals were usually standing and eating within 1 h after surgery. Ampicillin (2 g every day) was given intramuscularly for the first 3 days following surgery and after every experiment. Initial experiments were performed five days or more after surgery to allow for surgical recovery.

Physiological measurements

Fetal and maternal systemic blood pressures were continuously measured, except during arterial blood sampling, in all experiments by attaching the arterial catheters to Beckman low-volume displacement pressure transducers. Changes in both maternal uterine artery and fetal intra-abdominal umbilical artery blood flow were monitored continuously using the ultrasonic pulsed Doppler flow meter modified from the original design of Hartley and Cole (1974) and constructed by the University of Iowa Bioengineering Resource Facility (Haywood et al., 1981). The pulsed Doppler flow probe consisted of a Silastic cuff around a 1-mm-diam piezoelectric crystal that emits a 20-mHz ultrasonic signal. The same crystal receives the reflected signal from the passing blood cells in the intervals between ultrasonic pulses. The signals are then recorded either as pulsatile or mean flow velocity of a Doppler shift (kHz) on a Beckman Dynograph. The techniques for construction and use of the probes and application of the flow meter have been described in detail (Haywood et al., 1981; Van Orden et al., 1984). The electronic amplifiers in the system are extremely stable, and the changes in the velocity signals recorded from the flow probes are directly and reliably proportional to changes in the true volume flow (Haywood et al., 1981; Van Orden et al., 1984).

Drug induced changes in blood flow (BF) velocity of the maternal uterine artery and fetal intra-abdominal umbilical artery are expressed as a percentage of the control (% BF) and percent changes in vascular resistance (PCVR), using the Doppler flow probe, were calculated using the following formulas (Robillard et al., 1986):

$$\% \text{ BF} = [E_{DS}/B_{DS}] \times 100$$

$$\text{VR} = \text{mBP/DS}$$

$$\text{PCVR} = [(VR_E - VR_B)/VR_B] \times 100$$

E_{DS} is the Doppler shift (kH_z) during experimental periods (different doses of DOM); B_{DS} is the Doppler shift (kH_z) during the control period; VR is the vascular resistance (expressed arbitrarily in $mmHg/kH_z$); mBP is the mean systemic blood pressure (mmHg). DS is the Doppler shift (kH_z); and VR_E and VR_B are the VR during experimental and control periods, respectively.

Blood pressures and blood flows were recorded continuously on an eight-channel recorder (Beckman Type R Dynograph). Maternal and fetal heart rates were calculated from the pulsatile blood pressure tracing.

Arterial blood gas values were determined by removing 0.9 ml of blood anaerobically from the fetal or maternal arterial catheters into a 1.0-ml heparinized plastic syringe and analyzed for oxygen pressure (Po_2), pH, and carbon dioxide pressure (Pco_2), using a pH/blood gas analyzer (813, Instrumentation Laboratory). Base excess (BE) was calculated from Siggaard-Andersen curve nomogram incorporated in the blood gas analyzer.

Maternal rectal temperature was monitored by a scanning telethermometer (Model 47, Yellow Springs Instrument Co., Inc.).

DOM and ketanserin doses

DOM was obtained from the National Institute of Drug Abuse, Rockville, MD and ketanserin tartrate from Janssen, Beerse, Belgium. DOM was administered at 1, 2.5, 5, 10 or 20 $\mu g/kg$ of maternal weight and at 2.5, 5, 10, 20 and 40 $\mu g/kg$ after ketanserin 1 mg/kg administration.

These doses permitted quantifiable changes in fetal and maternal cardiovascular and arterial blood gas values without endangering the sheep preparation. Since the fetus grows rapidly from 100 days to term, we feel that the blood flow transducer will partially occlude blood flow as the fetus grows, thereby altering the blood flow hemodynamics. Therefore no more than two experiments within 2 weeks after surgery were performed on each ewe to minimize the reduction of umbilical artery blood flow through the Doppler flow probe due to fetal growth. The dose of DOM was dissolved in 3.0 ml of physiological saline (0.9%) and injected into the maternal jugular vein catheter over a 3-minute period. The dose of ketanserin was dissolved in 10 ml of distilled water and injected into the maternal jugular vein over a 10-minute period.

Experimental protocol

Each ewe and its fetus were monitored for a baseline period of 60 minutes before DOM administration in order to establish a baseline. During this period, arterial blood samples were withdrawn from the fetal and maternal arterial catheters 10 minutes before DOM administration in order to determine the baseline fetal and maternal blood gas values. The interval between any two doses of DOM was 40 minutes. Fetal and maternal arterial blood samples were withdrawn 20 minutes after each dose of DOM. MBP, FBP, MUABF and FUmBF were monitored throughout the experimental period following DOM administration. MHR and FHR were monitored at the peak of blood pressure response by increasing the speed of the recording paper and counting the pulses.

In order to study the blocking effect of ketanserin on the response produced by DOM, ketanserin (1 mg/kg) was infused over 15 minutes into

the maternal jugular vein catheter after the 60-minute period of calibration. Thirty minutes after infusion of ketanserin, different doses of DOM were injected into the maternal jugular vein catheter using the same injection sequence as for DOM alone. Cardiovascular monitoring was the same as above except that HR and blood gases were monitored at two additional periods, the first at the peak blood pressure response to ketanserin and the second 25 minutes after ketanserin infusion.

Data analysis

Regression lines were determined with the least-squares formula. Student's paired t test was employed to compare control to DOM treatment values and group t test was employed to compare DOM treatments before and after ketanserin. The term significant is used throughout to describe changes with a P value of less than 0.05. The results are presented as means ± SE.

Results

The mean (±SE) gestational age of ewes receiving DOM alone or ketanserin plus DOM were 124.4 ± 1.5 days and 122.8 ± 0.8 days, respectively. There was no significant difference in the gestational ages between the two groups. The control values for maternal and fetal cardiovascular parameters and arterial blood gas values for all studies obtained at 10 minutes before injection of DOM or ketanserin/DOM are presented in Table 1. A comparison of these control values indicated no significant differences between the two groups.

Table 1. Control fetal and maternal cardiovascular parameters and blood gas values prior to ewes receiving DOM or DOM plus ketanserin

	Fetal		Maternal	
Variable	DOM (n=5)	Ketanserin/DOM (n=8)	DOM (n=5)	Ketanserin/DOM (n=8)
mBP*(mmHg)	53.800 ± 1.10	51.000 ± 2.17	107.900 ± 5.00	99.700 ± 2.70
HR*(min^{-1})	158.300 ± 12.10	163.300 ± 6.70	110.000 ± 5.80	99.300 ± 9.10
pH	7.409 ± 0.01	7.385 ± 0.02	7.482 ± 0.02	7.494 ± 0.01
Pco_2*(mmHg)	42.400 ± 0.83	45.100 ± 1.29	32.200 ± 0.76	30.300 ± 1.40
Po_2*(mmHg)	17.900 ± 1.08	18.900 ± 1.44	90.300 ± 5.26	92.400 ± 2.59
BE*(mEq/l)	3.790 ± 0.90	3.250 ± 1.07	1.600 ± 1.41	1.480 ± 1.21
MRT*(°C)			40.000 ± 0.30	40.400 ± 0.20

*mBP indicates mean arterial blood pressure; HR, heart rate; Pco_2 arterial blood carbon dioxide partial pressure; Po_2, arterial blood oxygen partial pressure; BE, arterial blood base excess; MRT, maternal rectal temperature. Values are means ± SE.

Effects of DOM on arterial blood pressure and heart rate

Intravenous injections of DOM (1 μg/kg to 20 μg/kg) to the mother produced a dose-dependent rise in maternal and fetal arterial blood pressure (Fig. 1) which was accompanied by a fall in heart rate (Fig. 2). This was associated with an increase in pulse pressure. Peak blood pressure responses were obtained within 5 minutes following the administration of DOM (Fig. 3) and the duration of action was dependent upon the dose. The blood pressure returned to near control values within 30 minutes following the 20 μg/kg dose. There was a 2-3 minute delay in the increase of fetal arterial blood pressure following the increase of maternal arterial blood pressure. Tachyphylaxis to DOM in the doses used was not observed.

Fetal and maternal heart rate decreased immediately as the pressures rose. The maximal fall in fetal and maternal heart rates occurred at DOM 10 μg/kg even though the arterial blood pressures still increased at DOM 20 μg/kg. Fig. 4 illustrates the linear relationship between the fall in heart rate and the increase in mean arterial blood pressure. The significant difference in the slopes of the regression lines indicated that heart rate decrease in the fetus was more pronounced than that in the ewe. When arterial blood pressure increased 1 mmHg, FHR and MHR decreased 3.4 beats/min and 1.4 beats/min, respectively.

Effects of DOM on maternal uterine artery and fetal umbilical artery hemodynamics

A significant and dose-dependent decrease was observed in MUABF and FUmABF following DOM administration (Fig. 5). The threshold dose of DOM

Fig. 1. Dose-response curves for changes in the mean arterial blood pressure produced by intravenous administration of DOM to the mother in the conscious chronically instrumented pregnant ewe preparation. Solid lines indicate the results with DOM treatment before ketanserin administration. Dash lines indicate the results with DOM treatment after ketanserin administration. Ketanserin (1 mg/kg) was administrated intravenously to the ewe 30 minutes before beginning the administration of DOM. K indicates ketanserin. Results are illustrated as the $\bar{x} \pm$ SE. of 5 to 8 animals. Asterisk indicates the comparison of the result at each dose of DOM to the control. *, $P < 0.05$; ** $P < 0.01$. Cross indicates the comparison between the two results at the same dose of DOM before and after ketanserin administration. +, $P < 0.05$; ++, $P < 0.01$

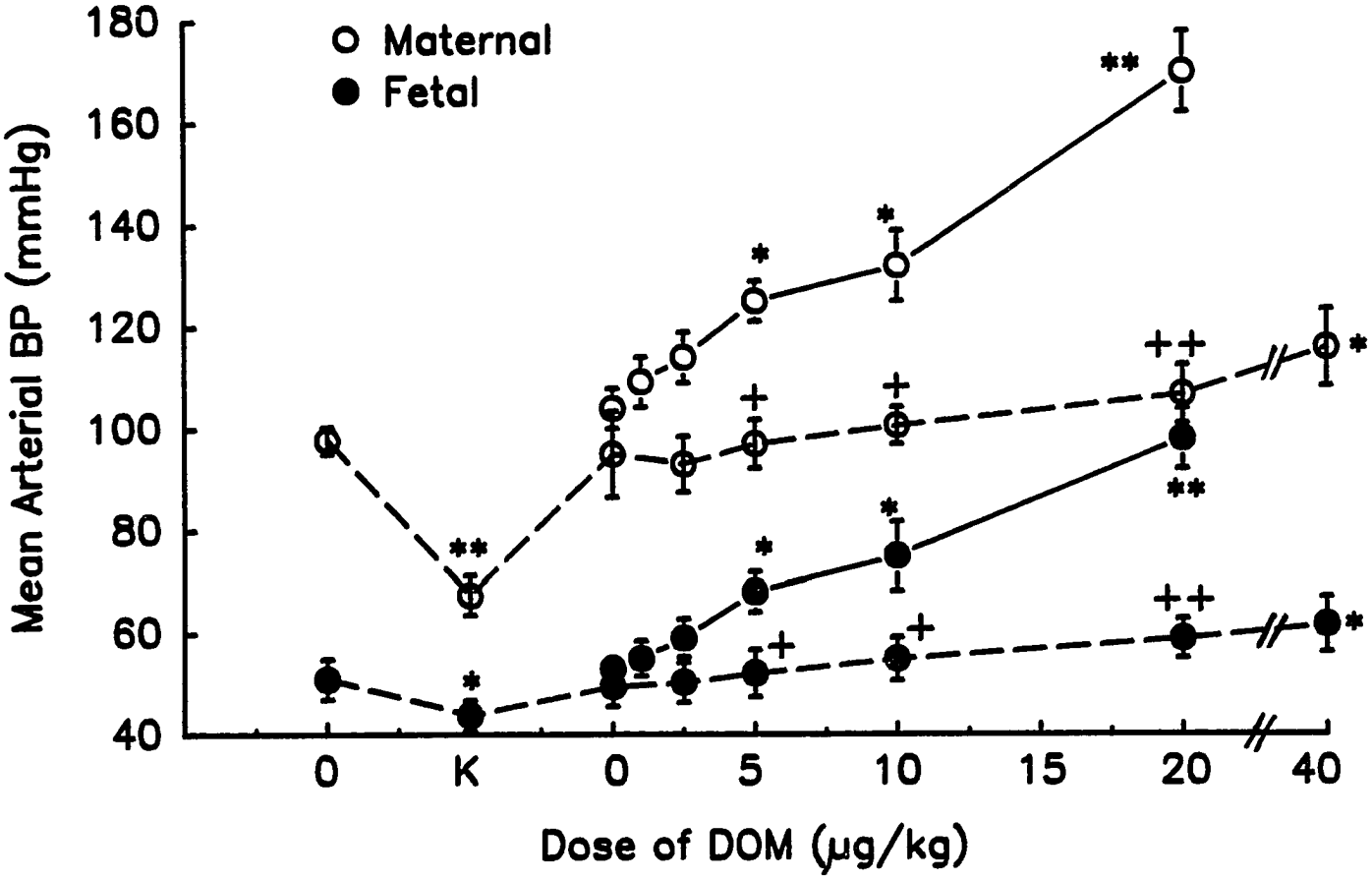

Maternal
Fetal
Mean Arterial BP (mmHg)
180
160
140
120
100
80
60
40
0
K
0
5
10
15
20
40
Dose of DOM (µg/kg)

Fig. 2. Dose-response curves for changes in the heart rate produced by intravenous administration of DOM to the mother in the conscious chronically instrumented pregnant ewe preparation. Solid lines indicate the results with DOM treatment before ketanserin administration. Dash lines indicate the results with DOM treatment after ketanserin administration. Ketanserin (1 mg/kg) was administrated intravenously to the ewe 30 minutes before beginning the administration of DOM. K indicates ketanserin. Results are illustrated as the $\bar{x} \pm$ SE. of 5 to 8 animals. Asterisk indicates the comparison of the result at each dose of DOM to the control. *, $P < 0.05$; **, $P < 0.01$. Cross indicates the comparison between the two results at the same dose of DOM before and after ketanserin administration. +, $P < 0.05$; ++, $P < 0.01$

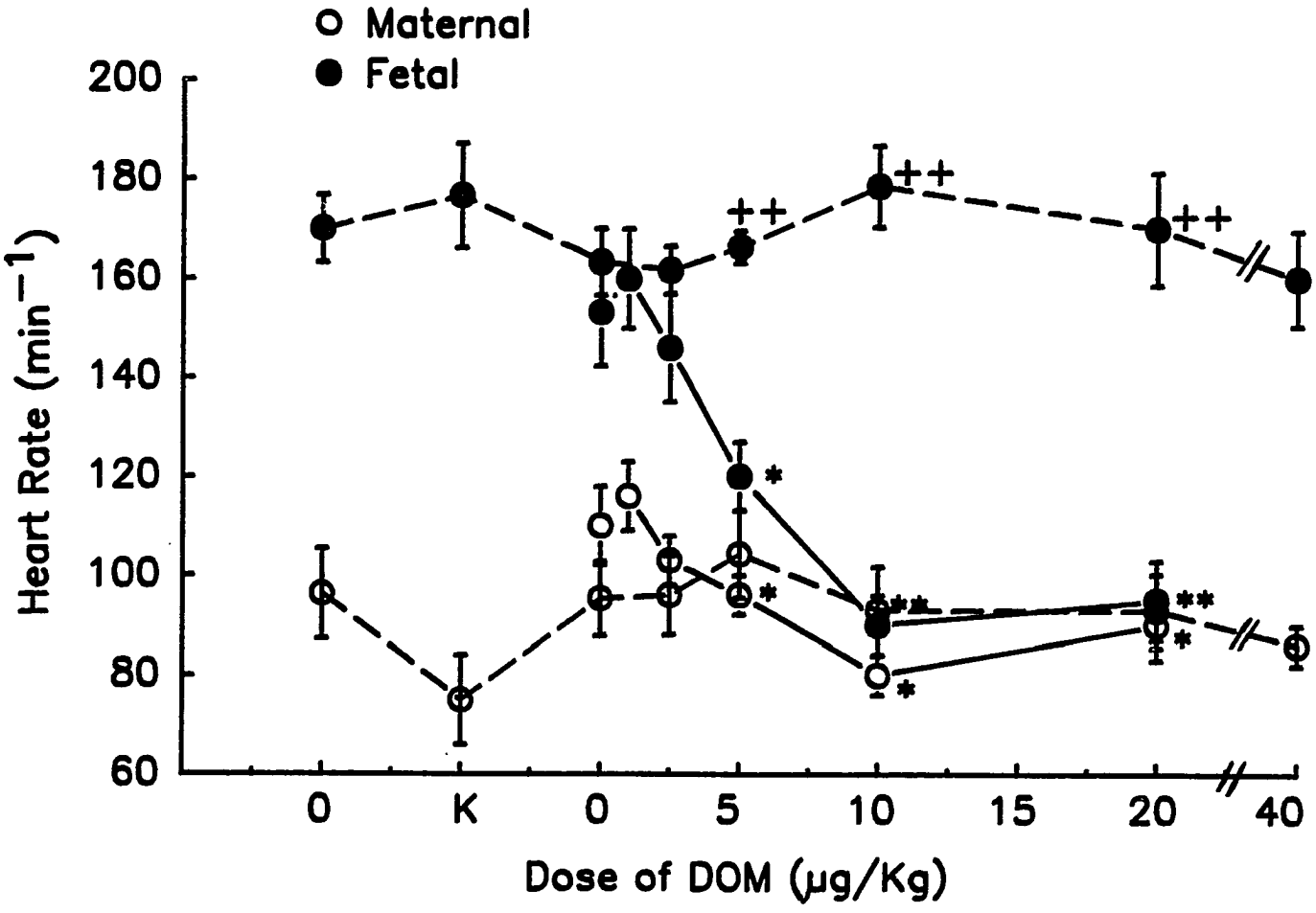

Maternal
Fetal
200
180
160
140
120
100
80
60
Heart Rate (min^{-1})
0
K
0
5
10
15
20
40
Dose of DOM (µg/Kg)
++
++
++
*
*
*
*
**
**
*

Fig. 3. Segment of a record showing the response of the maternal and fetal arterial blood pressure and the blood flow velocity of maternal uterine artery and fetal intra-abdominal umbilical artery to maternal intravenous administration of DOM (20 μg/kg) in the conscious chronically instrumented pregnant ewe preparation. DOM was injected at the arrow. The time scale is indicated at the bottom. The blood flow velocity was continuously monitored with the ultrasonic pulsed Doppler flow meter as described in Methods. The Doppler flow meter was calibrated for 0.5 volts per kH_z shift

Ewe 168

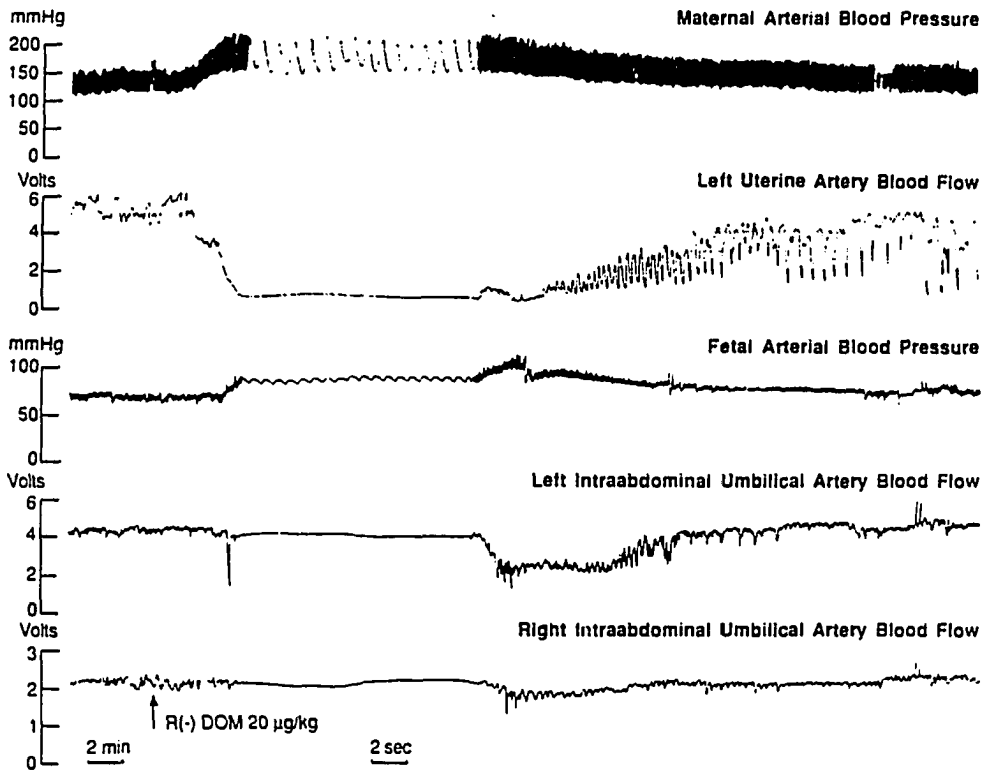

mmHg
200
150
100
50
0
Maternal Arterial Blood Pressure
Volts
6
4
2
0
Left Uterine Artery Blood Flow
mmHg
100
50
0
Fetal Arterial Blood Pressure
Volts
6
4
2
0
Left Intraabdominal Umbilical Artery Blood Flow
Volts
3
2
1
0
Right Intraabdominal Umbilical Artery Blood Flow
R(-) DOM 20 µg/kg
2 min
2 sec

Fig. 4. The relationship between an increase in the arterial blood pressure and a decrease in the heart rate for the conscious pregnant ewes and the fetal lambs when the arterial blood pressure was altered by intravenous administration of DOM to the ewe. The slopes of two regression lines were significantly different ($P < 0.05$)

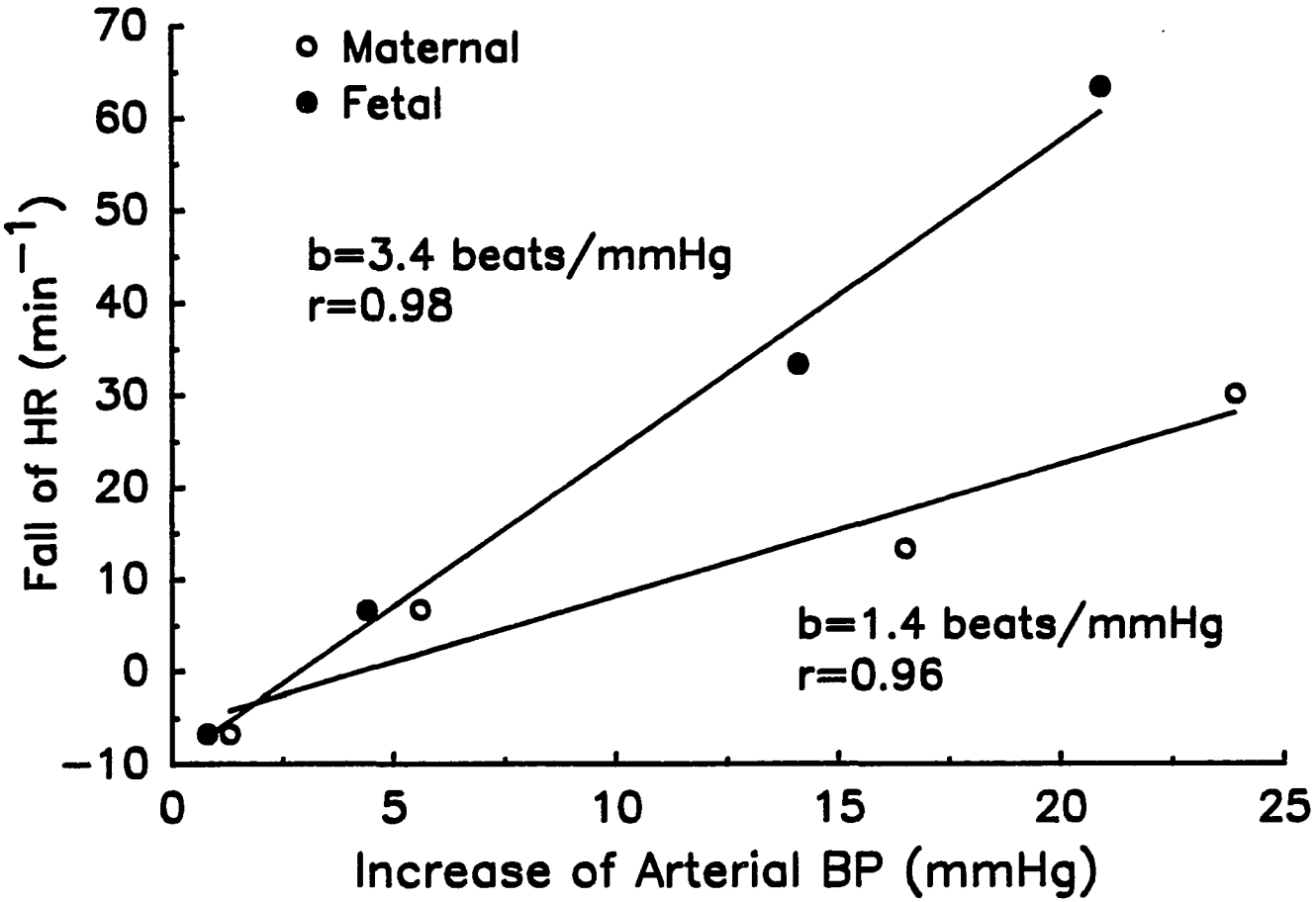
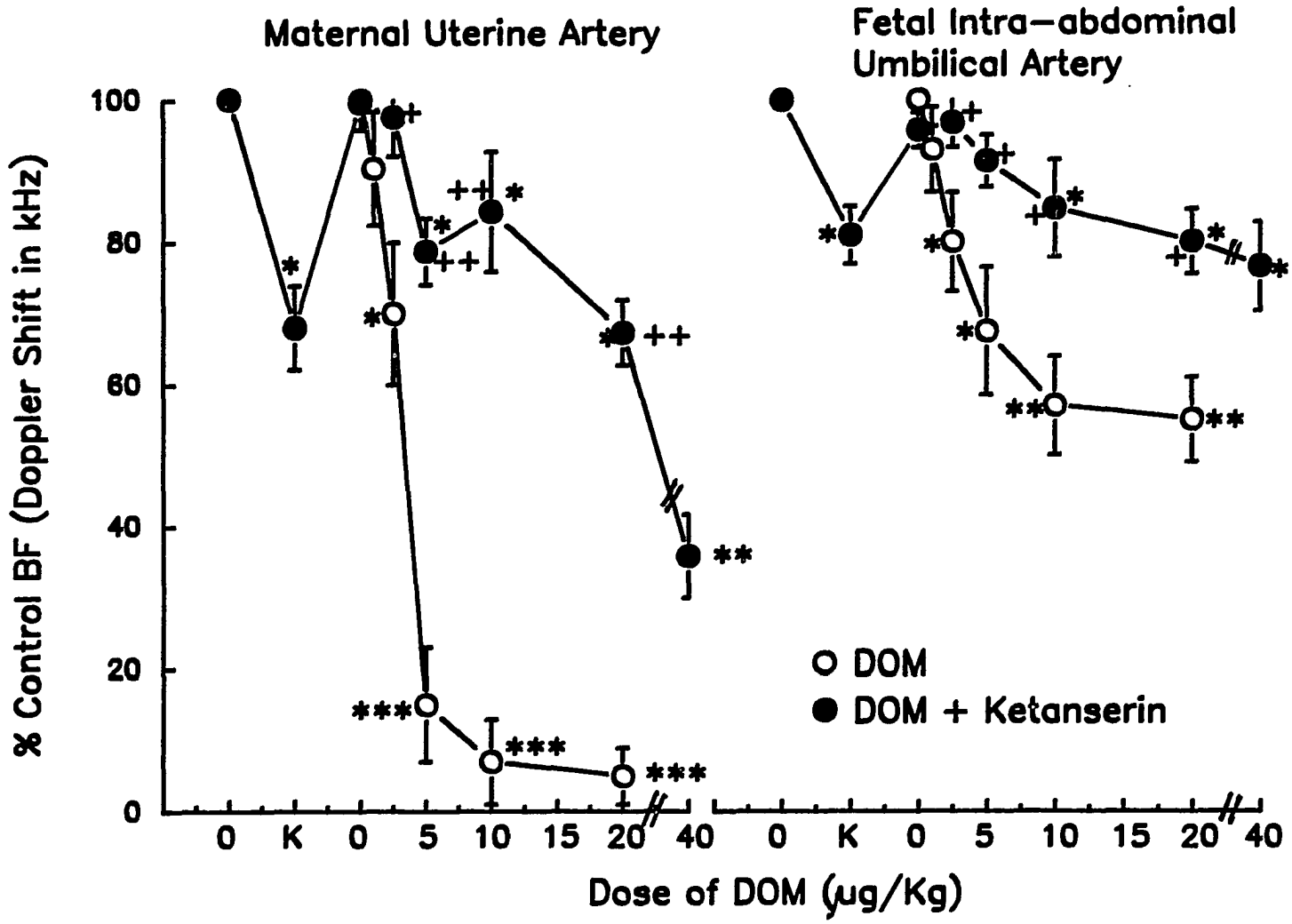

70
60
50
40
30
20
10
0
−10
0
5
10
15
20
25
Fall of HR (min^{-1})
Increase of Arterial BP (mmHg)
o Maternal
● Fetal
b=3.4 beats/mmHg
r=0.98
b=1.4 beats/mmHg
r=0.96

Fig. 5. Effects of intravenous administration of DOM to the mother, before and after ketanserin administration, on the blood flow (BF) of the maternal uterine artery and the fetal intra-abdominal umbilical artery in the conscious chronically instrumented pregnant ewe preparation. Ketanserin (1 mg/kg) was administrated intravenously to the ewe 30 minutes before beginning the administration of DOM. K indicates ketanserin. Results are illustrated as the $\bar{x} \pm$ SE. of 5 to 8 animals. Asterisk indicates the comparison of the result at each dose of DOM to the control. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$. Cross indicates the comparison between the two results as the same dose of DOM before and after ketanserin administration. +, $P < 0.05$; ++, $P < 0.01$


Maternal Uterine Artery
Fetal Intra–abdominal Umbilical Artery
% Control BF (Doppler Shift in kHz)
100
80
60
40
20
0
0 K 0 5 10 15 20 40
0 K 0 5 10 15 20 40
Dose of DOM (µg/Kg)
O DOM
● DOM + Ketanserin

to produce these decreases was 2.5 μg/kg. The peak MUABF response was obtained at about the same time as the peak MBP response. The duration of action was dependent upon the dose and was clearly associated with the MBP response. The peak FUmABF response occurred after a 2-3 min delay from administration, and it correlated with the peak FBP response. The duration of the FUmABF response was shorter than the MUABF response. A maximal reduction of 92% in MUABF was observed within 5 minutes after DOM (20 μg/kg) and this peak response lasted for about 30 sec. The MUABF tracing then developed a spasmodic pattern and this lasted for more than an hour (Fig. 3). The maximal reduction in FUmABF was only 40% and the spasmodic pattern of blood flow was not observed.

Reflecting the changes in mean blood pressure and blood flow, DOM significantly and dose-dependently increased both maternal uterine artery (Fig. 6) and fetal umbilical artery (Fig. 7) vascular resistance. However, the change in vascular resistance was always smaller in the fetal umbilical artery than in the maternal uterine artery. The maximal rise in vascular resistance (19.6 ± 1.7 - fold) in the maternal uterine artery was observed within 5 minutes after DOM (20 μg/kg) which was significantly ($P < 0.01$) greater than that in the fetal umbilical artery (2.6 ± 0.4 - fold).

Effects of DOM on arterial blood gas and pH values and on the maternal rectal temperature

In all dosage groups, maternal blood gas values (Po_2, Pco_2 and pH) did not vary significantly from baseline values after DOM administration. The responses of fetal pH, Pco_2, Po_2 and BE to maternally administered DOM are presented in Table 2. A significant

Fig. 6. Effects of intravenous administration of DOM to the mother, before and after ketanserin administration, on the maternal uterine artery resistance in the conscious chronically instrumented pregnant ewe preparation. Ketanserin (1 mg/kg) was administrated intravenously to the ewe 30 minutes before beginning the administration of DOM. K indicates ketanserin. PCVR indicates percent change in vascular resistance calculated as described in Methods. Results are illustrated as the $\bar{x} \pm$ SE. of 5 to 8 animals. Asterisk indicates the comparison of the result at each dose of DOM to the control. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$. Cross indicates the comparison between the two results at the same dose of DOM before and after ketanserin administration. ++, $P < 0.01$; +++, $P < 0.005$

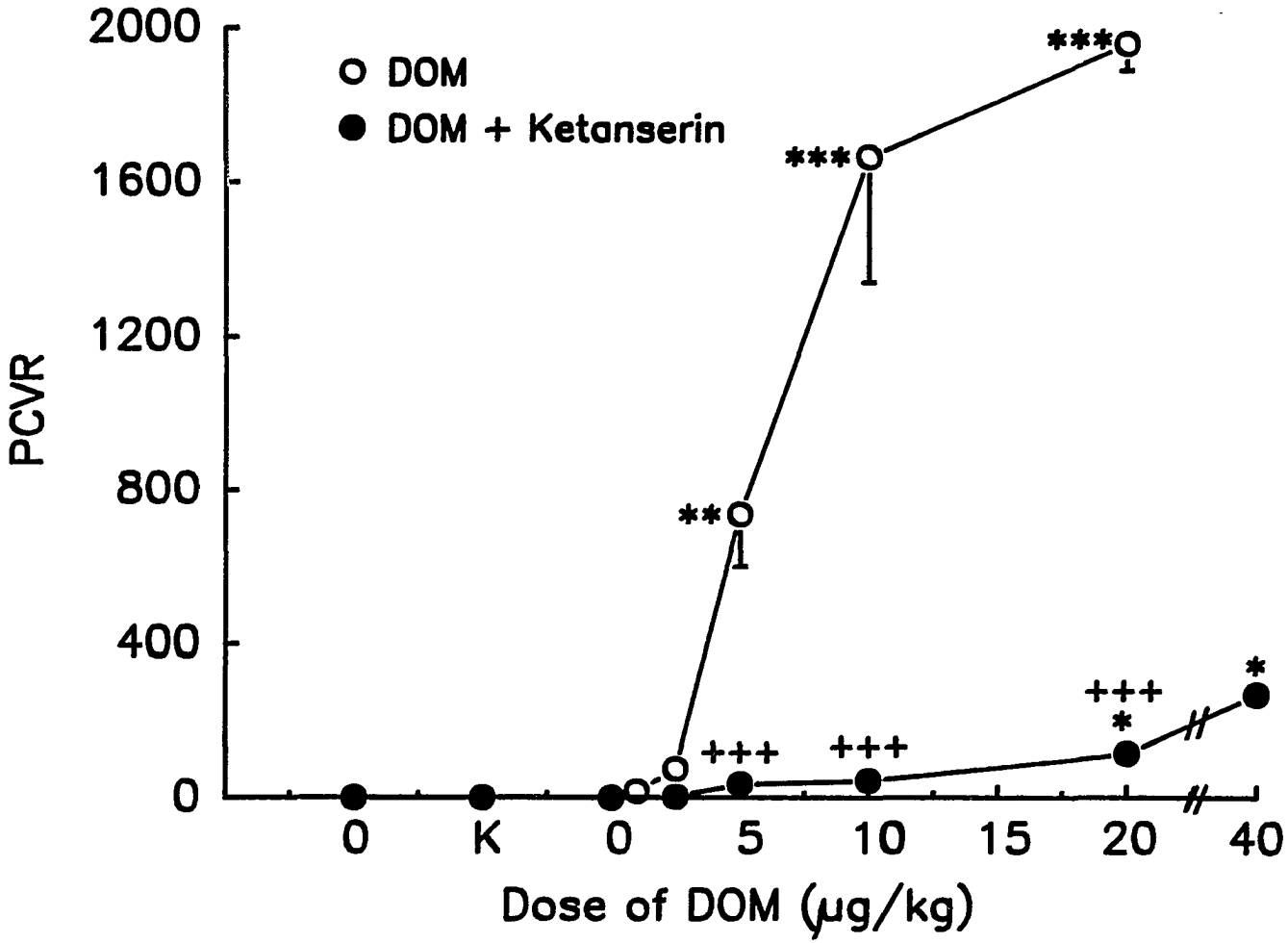

2000
1600
1200
800
400
0
PCVR
DOM
DOM + Ketanserin
***
***
**
+++
+++
+++
*
*
0
K
0
5
10
15
20
40
Dose of DOM (µg/kg)

Fig. 7. Effects of intravenous administration of DOM to the mother, before and after ketanserin administration, on the fetal intra-abdominal umbilical artery resistance in the conscious-chronically instrumented pregnant ewe preparation. Ketanserin (1 mg/kg) was administrated intravenously to the ewe 30 minutes before beginning the administration of DOM. K indicates ketanserin, PCVR indicates percent change in vascular resistance calculated as described in Methods. Results are illustrated as the $\bar{x} \pm$ SE. of the 5 to 8 animals. Asterisk indicates the comparison of the result at each dose of DOM to the control. * < 0.05; **, $P < 0.01$. Cross indicates the comparison between the two results at the same dose of DOM before and after ketanserin administration. +, $P < 0.05$, ++, $P < 0.01$

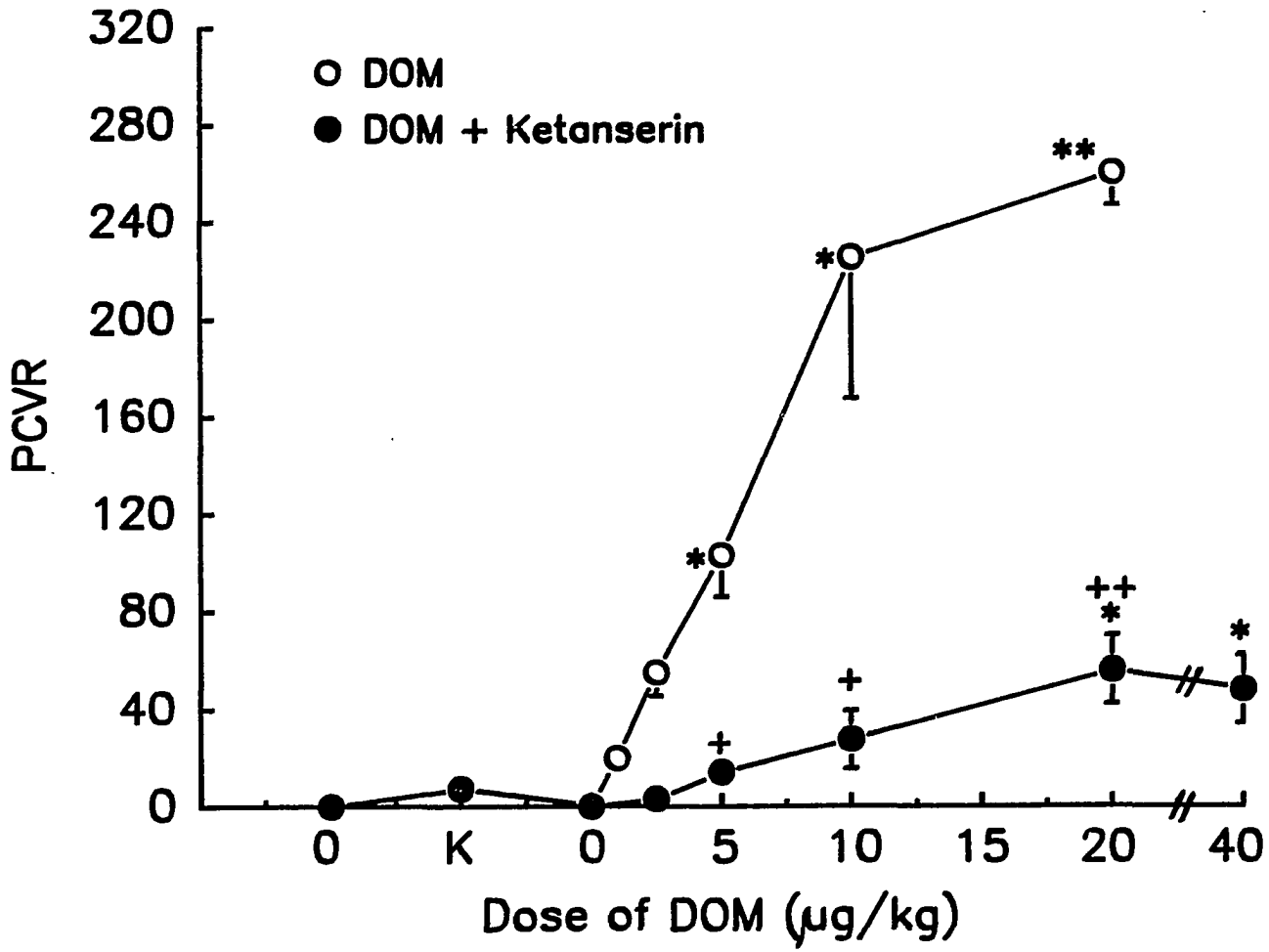

320
280
240
200
160
120
80
40
0
PCVR
DOM
DOM + Ketanserin
**
*
*
++
*
+
+
*
0
K
0
5
10
15
20
40
Dose of DOM (µg/kg)

Table 2. Effects of DOM pre and/or after ketanserin administration on fetal arteri

DOM dose (μg/kg)	Pre ketanserin (n = 5)				After ketanse	
	pH	Pco_2[a](mmHg)	Po_2[a](mmHg)	BE[a](mEq/l)	pH	Pco_2(mmHg)
0.0	7.409±0.01	42.4±0.83	17.9±1.08	+3.79±0.90	7.385±0.02	45.1±1.29
2.5	7.400±0.02	43.2±1.00	17.4±1.07	+3.46±1.45	7.378±0.02	44.6±1.10
5.0	7.383±0.01	45.1±0.90*	17.5±0.51	+3.30±1.37	7.375±0.01	46.0±1.20
10.0	7.370±0.02*	45.6±1.05*	15.3±1.09*	+2.70±1.15	7.366±0.02	46.8±1.60
20.0	7.335±0.01*	47.9±1.10*	14.0±0.90*	+1.30±0.40*	7.345±0.02*	48.3±1.40
40.0					7.324±0.03*	50.3±2.10*

[a]Pco_2 indicates carbon dioxide partial pressure; Po_2, oxygen partial pres
Values are means ± SE.
*Changes are significantly different from the control values ($P<0.05$).

or after ketanserin administration on fetal arterial blood values

erin (n = 5)			After ketanserin (n = 8)		
Po_2[a](mmHg)	BE[a](mEq/l)	pH	Pco_2(mmHg)	Po_2(mmHg)	BE(mEq/l)
17.9±1.08	+3.79±0.90	7.385±0.02	45.1±1.29	18.9±1.44	+3.25±1.07
17.4±1.07	+3.46±1.45	7.378±0.02	44.6±1.10	18.9±1.60	+2.48±1.00
17.5±0.51	+3.30±1.37	7.375±0.01	46.0±1.20	20.0±1.80	+2.85±0.90
15.3±1.09*	+2.70±1.15	7.366±0.02	46.8±1.60	19.5±1.60	+2.60±0.80
14.0±0.90*	+1.30±0.40*	7.345±0.02*	48.3±1.40	17.7±1.60	+1.86±0.80*
		7.324±0.03*	50.3±2.10*	16.5±2.10*	+1.35±1.50*

loxide partial pressure; Po_2, oxygen partial pressure; BE, base excess.

ly different from the control values ($P<0.05$).

reduction ($P < 0.05$) in pH was observed 20 minutes after maternal DOM doses of 10 and 20 μg/kg. Pco_2 significantly increased at the doses 5, 10 and 20 μg/kg, while a reduction in BE was observed at the 20 μg/kg dose. Po_2 decreased to 15.3 ± 1.09 and 14.0 ± 1.07 after DOM administration of 10 and 20 μg/kg, respectively, which were significantly different ($P < 0.05$) from the control of 17.9 ± 1.08 (Table 2).

There were no changes in maternal rectal temperature 20 minutes after maternal DOM administration at all the doses studied.

Effect of ketanserin on arterial blood pressure and heart rate responses to DOM

Ketanserin, infused into the maternal jugular vein at 1 mg/kg over a 15-minute period, produced a fall in MBP from 99.7 ± 2.7 mmHg to 69.4 ± 3.9 mmHg ($P < 0.01$) (Fig. 1). The peak reduction of MBP was obtained within 5 minutes after the infusion of ketanserin. MBP returned to baseline values by 25 minutes. MHR was 78 ± 15 after ketanserin infusion and was not significantly different from the baseline value ($P > 0.05$) (Fig. 2). The fall in FBP from 50.9 ± 2.2. mmHg to 43.8 ± 2.0 ($P < 0.05$) was less than that in the mother (Fig. 1). No significant changes were observed in FHR after ketanserin infusion (Fig. 2).

Thirty minutes after ketanserin infusion, MBP and FBP responses to DOM (5, 10 and 20 μg/kg) were significantly ($P < 0.05$) inhibited (Fig. 1). A significant ($P < 0.05$) increase in MBP and FBP was only observed at the higher dose of DOM (40 μg/kg). MHR and FHR did not vary significantly from baseline values at any dose of DOM after ketanserin (Fig. 2).

Effect of ketanserin on maternal uterine artery and fetal umbilical artery hemodynamic responses to DOM

Following the infusion of ketanserin (1 mg/kg) there was a decrease in MUABF of 32 ± 5.9% ($P < 0.05$) (Fig. 5) which corresponded to the reduction in MBP. MUABF and MBP returned to baseline values at the same time. The reduction in FUmABF (19 ± 4.1%) was significant ($P < 0.05$) but less than the reduction in MUABF (Fig. 5). Since ketanserin decreased both arterial blood pressure and blood flow simultaneously, there were no significant changes in vascular resistances in the maternal uterine artery and the fetal umbilical artery to ketanserin.

Thirty minutes after ketanserin infusion, MUABF was still decreased after DOM at 5, 10, 20, 40 μg/kg ($P < 0.05$) (Fig. 5). However, the reductions were significantly less ($P < 0.01$) than those in MUABF to DOM without ketanserin (Fig. 5), indicating the reduction in MUABF to DOM was inhibited by ketanserin. In the presence of ketanserin, DOM, in doses of 10, 20 and 40 μg/kg, produced a linear reduction in MUABF (Fig. 5). DOM (10, 20 and 40 μg/kg) produced a significant reduction ($P < 0.05$) in FUmABF after ketanserin administration but there was no significant difference between the doses of DOM. The decrease in FUmABF to DOM (2.5, 5, 10 and 20 μg/kg) in the presence of ketanserin (1 mg/kg) was significantly less than that to the same doses of DOM in the absence of ketanserin ($P < 0.05$) (Fig. 5).

Since both an increase in MBP and reduction in MUABF to DOM were blocked by ketanserin, Fig. 6 shows that the changes in vascular resistance in the maternal uterine artery to DOM in the presence of ketanserin was much less ($P < 0.01$) than that to the same dose of DOM in

the absence of ketanserin. There was a linear increase ($P < 0.05$) in vascular resistance in the maternal uterine artery at DOM doses of 10, 20 and 40 μg/kg after ketanserin administration (Fig. 6). The changes in vascular resistances in the fetal umbilical artery to DOM with ketanserin was also less ($P < 0.05$) than that to DOM without ketanserin (Fig. 7). The increase in vascular resistance in the fetal umbilical artery reached its plateau at the DOM dose of 20 μg/kg after ketanserin administration (Fig. 7).

Effect of ketanserin on arterial blood gas and pH responses to DOM

Maternal and fetal arterial blood gas values (Po_2, Pco_2, BE and pH) did not vary significantly from baseline values after ketanserin infusion. The reduction in fetal arterial blood pH to DOM 10 μg/kg was blocked by ketanserin (Table 2). DOM (20 μg/kg) still produced a reduction ($P < 0.05$) in fetal pH, but the change was less ($P < 0.05$) than that to the same dose of DOM without ketanserin. With a higher dose of DOM (40 μg/kg), fetal pH was further decreased (Table 2). No significant changes in fetal arterial blood Pco_2 and Po_2 were observed to DOM in doses up to 20 μg/kg after ketanserin administration. A higher dose of DOM (40 μg/kg) produced an increase on fetal Pco_2 and decrease in Po_2 (Table 2). No inhibitory effect of ketanserin on the reduction in BE to DOM (20 μg/kg) was observed (Table 2).

Discussion

The results of this study indicate that maternally administrated DOM produces dose-dependent increases in ovine maternal and fetal blood

pressures accompanied by a fall in heart rate. Ketanserin, a selective 5-HT_2 receptor antagonist (Leysen et al., 1981; Leysen et al., 1982; Van Nueten et al., 1981), inhibited the pressor response to DOM, indicating that 5-HT_2 receptor stimulation was involved in the response to DOM. This observation is in accord with previous reports involving rats, dogs and cats which indicate that the DOM-induced increase in blood pressure was mediated via serotonin receptor stimulation (Huang and Ho, 1972; Cheng et al., 1973; Tadepalli et al., 1975). It has been demonstrated that DOM is a potent agonist at 5-HT_2 receptors both in the central nervous system (Glennon et al., 1983, 1984) and in the periphery (Dyer, 1983; Zhang and Dyer, 1989a, 1989b). 5-HT_2 receptors mediate vasoconstriction (Bradley et al., 1986; Cohen et al., 1983, Humphrey, 1984; Leysen et al., 1984; Maayani et al., 1984; Van Nueten et al., 1981, 1984) and since DOM selectively activates 5-HT_2 receptors, DOM always produces a pressor effect. In the present study, ketanserin produced a transitory decrease in maternal and fetal blood pressure without affecting the heart rate. The transitory decreases in the maternal uterine artery and fetal umbilical artery blood flow following ketanserin administration were probably reflected by the decrease in the blood pressure. Ketanserin has been shown to reduce blood pressure in both animals (Van Nueten et al., 1981) and hypertensive humans (DeCree et al., 1981) without causing a compensatory increase in heart rate. However, there is a controversy as to whether the hypotensive effect of ketanserin rests with the blockade of α_1- or 5-HT_2-receptors or with both (Humphrey et al., 1982; Vanhoutte and Van Nueten, 1983; Fozard, 1983; Janssen, 1983). Since ketanserin can block the pressor response

mediated by α-receptors (Fozard, 1982; Kalkman et al., 1982; Nelson et al., 1984), alpha-receptor stimulation in the pressor response to DOM can not be ruled out in this study. However, Cheng et al. (1973) and Tadepalli et al. (1975) demonstrated that the pressor response to DOM was not mediated by α-receptor stimulation in dogs and cats since phentolamine did not block this response. The pressor response to DOM was not elicited by increasing myocardial activity (cardiac output) nor inhibition of the vagus (Tadepalli et al., 1975). It has been reported that DOM could release 5-HT from platelets in rabbits and dogs (Cheng et al., 1973). Therefore, some of the pressor effects produced by DOM could be contributed by 5-HT.

The significance of dose-dependent decreases in uterine and umbilical blood flow was defined more clearly in terms of vascular resistance. Alterations in blood flow to a vascular bed may result from changes in perfusion pressure or changes in vascular resistance from vasoconstriction or vasodilatation. In response to maternally administrated DOM, there was a concurrent rise in maternal blood pressure and a decrease in uterine blood flow, reflecting a dramatic increase in vasoconstriction of the uterine artery. The vaso-constriction effect of DOM was antagonized by ketanserin. This is in accord with our recent finding that DOM produced a potent vasoconstriction on the isolated uterine artery from late pregnant sheep with the dissociation constant being 1.8×10^{-7}M (Zhang and Dyer, 1989a). Ketanserin shifted the dose-response curves of DOM in a parallel manner to the right with the pA_2 value being 8.33, indicating uterine artery vasoconstriction produced by DOM was mediated by 5-HT_2

receptors. The reduced vasoconstriction observed in the fetal umbilical artery as compared to the uterine artery is unexpected since DOM was a more potent agonist in isolated umbilical arteries than in isolated uterine arteries (Zhang and Dyer, 1989a, 1989b). One of possibilities is that DOM is not crossing the placenta in sufficient concentration to elicit umbilical artery vasoconstriction. This is supported by preliminary experiments which indicated that if DOM was directly given to the fetus through a femoral vein catheter, there was a dramatic increase in umbilical artery vascular resistance. In the absence of measured DOM concentrations in maternal or fetal blood in this study, the relative portion of the effect of DOM which is a direct action on the fetal circulation remains speculative.

Tachyphylaxis to the effects of DOM was not observed in this study. This observation does not agree with those by Friedman and co-workers (1978) or of Tadepalli et al. (1975). They found that tachyphylaxis to the blood pressure response to the second dose of DOM developed rapidly and was present up to at least 2 hrs following the first DOM injection. The third response was less than 30% of the first response. Marked tachyphylaxis to DOM has also been reported to occur for its hallucinatory effect in man (Angrist et al. 1974) and to its behavioral and EEG effects in cats (Wallach et al. 1972). However, vascular reactivity following DOM remained the same and tachyphylaxis was not observed (Dyer et al., 1973; Dyer, 1983; Zhang and Dyer, 1989b). The mechanism of tachyphylaxis development is not clear. The difference between this study and those of Friedman et al. (1978) and Tadepalli et al. (1975) could result from the different route of administration

and/or the different dose used or the use of different species. The intraventricular administration of DOM was used by Friedman et al. (1978) and Tadepalli et al. (1975) while intravenous administration was used in this study. The higher dose of DOM (1-4 mg/kg) used by Tadepalli et al. (1975) may also have contributed to the development of tachyphylaxis.

The bradycardia which occurred following DOM administration appeared to be mediated reflexively in response to the increased blood pressure. The finding that the decrease in fetal heart rate was more pronounced than that in the ewe is of interest. The existence of arterial baroreceptor reflexes in the fetus (0.6-1.0 gestation) is well established (Shinebourne et al., 1972; Maloney et al., 1977). The degree of slowing of the heart rate in response to a rise in arterial pressure varied between the results reported by these authors. Shinebourne et al. (1972) found no difference between the fetus and the neonate, whereas Maloney et al. (1977) found a reduced bradycardia to increase in blood pressure in the newborn as compared to the fetus. Using a balloon to alter arterial pressure, Dawes et al. (1980) found that there was a consistent increase of baroreflex sensitivity from the fetus to the adult. In our study, the decrease in fetal heart rate could be caused by the decreased blood flow in the maternal uterine artery as well as the increase in the fetal blood pressure.

The physiological importance of the heart rate stems from the fact that it is one of the two components of the cardiac output, the other being the stroke volume. Since the two ventricles effectively function parallel with the fetus, the Frank-Starling mechanism would not be of

particular importance. Because of this limited ability for increasing stroke volume, fetal cardiac output is regulated predominantly by changes in heart rate (Rudolph and Heymann, 1974). The fast heart rate is an important mechanism which provides the fetus with the high cardiac output necessary to meet its metabolic activities. In general, sustained bradycardia is thought to reflect fetal distress. It has been found that the unanesthetized fetal lamb consistently developed bradycardia coupled with an increase in the systematic arterial pressure when fetal arterial Po_2 was lowered (Cohn et al., 1972). In the present study, vasoconstriction by DOM of the maternal uterine artery reduced oxygen delivery to the fetus and impaired fetal oxygenation. It is well established that norepinephrine (NE) concentration increases in the fetal plasma during acutely induced fetal hypoxia (Cohen et al., 1982; Lewis et al., 1982, 1984). Under resting conditions, small variations in oxygen tension may be an important modulator of NE release. Cheung and Brace (1988) showed that NE increased 41 pg/ml for each 1-mmHg decrease in oxygen tension. The high NE concentration found during fetal hypoxia tends to produce a progressive increase in arterial pressure and a decrease in heart rate. Vasopressin may also be involved in the fetal bradycardia and hypertension since it has been shown that arginine vasopressin (AVP) is increased in the fetal plasma during hypoxia (Rurak, 1978; Stark et al., 1982; Stegner et al., 1984; Tomita et al., 1985).

The direct pressor effect of DOM on umbilical vasculature may also contribute to an increase in fetal blood pressure and the fall in the heart rate. The umbilico-placental circulation of the fetus is the

major factor which contributes to the low fetal systemic vascular resistance. This part of the fetal vascular bed accounts for about two-thirds of the fetal cardiac output and is regarded as a low resistance network grafted in parallel with the circulation of the body of the fetus (Assali et al., 1968). Therefore, a small increase in umbilical vasculature resistance would promptly increase the systemic vascular resistance and produce an increase in arterial blood pressure and a decrease in cardiac output. It has been demonstrated that compression of the whole umbilical cord of the fetal lamb produced a bradycardia along with hypertension (Assali et al., 1968). We have demonstrated that DOM is a potent vasoconstrictor on the isolated umbilical vasculature of the fetal lamb and the vasoconstriction is mediated via 5-HT_2 receptors (Zhang and Dyer, 1989b). The dissociation constant of DOM in constriction of the umbilical artery of the fetal lamb was 36nM (Zhang and Dyer, submitted for publication). In the present study, DOM (10 μg/kg) increased the umbilical artery resistance about 2.3 times, which was less than it did on the maternal uterine artery (about 17 times). However, as discussed above, the reason for the increased response of the uterine vasculature over that of the umbilical vasculature is likely due to the fact that the drug was directly introduced to the systemic circulation of the mother. Therefore, the concentration of DOM was higher at the level of the uterine vasculature than that of the umbilical vasculature since it would need to diffuse across the placenta in order to produce vasoconstriction of the umbilical artery.

Mild hypoxia per se may not depress myocardial function. However,

a combination of hypoxia and acidemia produced marked depression of myocardial performance (Rudolph and Heymann, 1974). The combined metabolic and respiratory acidosis which developed in the fetus after the maternal administration of DOM could impair fetal brain function as well as myocardial performance. Ketanserin inhibited the fall in Po_2 and pH in the fetus in response to the maternal administration of DOM and probably due to its inhibitory effect on maternal uterine vasoconstriction produced by DOM.

In summary, DOM, administered to the pregnant ewe, produced a dose-dependent increase in maternal and fetal arterial pressure accompanied by a decrease in heart rate. The maternal uterine blood flow dramatically decreased following DOM (5 to 20 μg/kg), which indicated a significant increase in vasoconstriction of the uterine artery. The fetus was distressed after maternal administration of DOM. Hypoxia and combined metabolic and respiratory acidosis were developed in the fetus after maternal DOM injection. A significant proportion of the fetal cardiovascular responses were probably produced by an indirect effect of DOM on the maternal uterine artery which in turn impaired the oxygenation of the fetus. Ketanserin inhibited the effects of DOM on the cardiovascular system of the ewe and fetus and on changes in blood gas values in the fetus, indicating the involvement of 5-HT_2 receptors in these responses. These observations suggest that the administration or consumption of 5-HT_2 receptor agonists during pregnancy may be hazardous to the fetus.

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SECTION II. CHARACTERIZATION OF 5-HYDROXYTRYPTAMINE (5-HT) RECEPTORS ON ISOLATED OVINE UTERINE ARTERY IN LATE PREGNANCY[1]

Abstract

5-Hydroxytryptamine (5-HT) and 2,5-dimethoxy-4-methyl-amphetamine (DOM) are potent agonists on isolated ovine uterine arteries in late pregnancy. Similar pA_2 values (8.56 and 8.33, respectively) of ketanserin, tested against 5-HT and DOM, indicate that responses produced by both agonists are mediated by the 5-HT_2 receptor. The contractions produced by 8-OH-DPAT (8-hydroxy-dipropylaminotetralin) and 2-methyl-5-HT were also blocked by ketanserin (10^{-8}M) with the dissociation constants (K_B) being 2.49 nM and 2.88 nM, respectively. This provides evidence that these agonists are activating 5-HT_2 receptors in the ovine uterine artery. DOM was more potent than 5-HT, but had a similar efficacy to that of 5-HT. The greater affinity of DOM may explain its greater potency. The dissociation constants (K_A) of 5-HT and DOM acting on 5-HT receptors were determined by analysis of concentration-response data before and after fractional inactivation of receptors with dibenamine. The mean K_A values for 5-HT and DOM were 3.7 $\pm$ 0.7 x 10^{-7}M and 1.8 $\pm$ 0.3 x 10^{-7}M, respectively. Assessment of receptor occupancy vs. functional response demonstrated little or no receptor reserve in this tissue. Several other 5-HT receptor agonists caused contractions but were much less potent than 5-HT. The order of

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[1]Submitted to J. Pharmacol. Exp. Ther. as a research paper by Lubo Zhang and Donald C. Dyer.

potency of these agonists was determined to be DOM > 5-HT ≥ α-methyl-5-HT >> 8-OH-DPAT > 2-methyl-5-HT > 1-(3-chlorophenyl) piperazine (mCPP) > m-trifluoromethyl-phenylpiperazine (TFMPP). The presence of 5-HT_{1A} receptor in this tissue is unlikely since ketanserin antagonized the contractile response to 8-OH-DPAT. 1-(2-methoxyphenyl) piperazine (2-MPP) lacked agonist activity and TFMPP and mCPP had very weak agonist activities. All three phenylpiperazine derivatives effectively blocked contractile responses to 5-HT. The dissociation constants (K_B) were determined to be: mCPP, 0.13 μM; TFMPP, 0.22 μM; 2-MPP, 1.43 μM. The potent and selective 5-HT_3 receptor antagonist MDL 72222 was without effect against 5-HT, indicating that the uterine artery 5-HT receptor is not of the 5-HT_3 subtype. Activation of α-adrenergic receptors was not involved in contractions to 5-HT.

Introduction

Various blood vessels contract upon exposure to 5-hydroxytryptamine (5-HT). One of the vascular beds which has received little study concerning 5-HT receptors is that of the uterus, especially in pregnancy. Dyer and Gough (1971) observed that 5-HT has primarily excitatory actions on isolated human uterine arteries. At lower concentrations, 5-HT and norepinephrine (NE) were equally active. Since uteroplacental exchange can be altered by drugs acting on either uterine or umbilical placental blood vessels as well as on uterine muscle (Meschia, 1983), it is important to have knowledge of drug responses and drug receptors on uterine blood vessels.

The pregnant ewe is widely used as an experimental animal in the study of fetal physiology/pharmacology and since we are currently investigating the effects of serotonergic agonists in the chronically instrumented ewe/fetus model, we found it desirable to study the effects of 5-HT agonists/antagonists on isolated uterine arteries. This approach permits a more detailed investigation of receptor mechanisms. In addition, our previous studies on isolated umbilical vasculature were carried out on tissues obtained at term or within two weeks of term (Dyer, 1970; Gant and Dyer, 1971). Therefore, the investigation of 5-HT receptor mechanisms in uterine artery near term will permit us to correlate these data with our previous work on umbilical vasculature and our ongoing investigation of serotonergic receptor mechanisms in the near term chronically instrumented ewe/fetus model.

The original classification of 5-HT receptors proposed by Gaddum and Picarelli (1957) and modified by Peroutka and Snyder (1979) continues to evolve (Bradley et al., 1986). The characterization of 5-HT receptors with the corresponding pharmacological responses has been investigated in various tissues, including blood vessels. Vasoconstriction in many arteries has been found to be mediated by a receptor similar to the 5-HT_2 binding site (Van Nueten et al., 1981, 1984; Leysen et al., 1984; Maayani et al., 1984). The classification of 5-HT_1 receptors in tissues is complicated since at least four subtypes have been proposed and because highly selective antagonists are not available (Pedigo et al., 1981; Pazos et al., 1984; Heuring and Peroutka, 1987). This is in contrast with that of 5-HT_2 or 5-HT_3 receptors for which selective antagonists exist.

Knowing the dissociation constants of 5-HT receptor agonists and competitive antagonists acting on uterine blood vessels would provide fundamental information necessary for the eventual subtyping of 5-HT receptors in this tissue and permit a comparison to other tissues. In the present study, the potency order of 5-HT, 2,5-dimethoxy-4-methyl-amphetamine (DOM) and other serotonergic agonists acting on the ovine uterine artery obtained in late pregnancy has been determined. The apparent dissociation constants were obtained for 5-HT, DOM and several competitive 5-HT receptor antagonists in this tissue.

Methods

Adult pregnant mixed breed sheep near term were anesthetized with pentobarbital and then exsanguinated. The pentobarbital was administered via the external left jugular vein. An incision in the abdomen was made and the uterus exposed. The left main uterine artery was isolated and removed without stretching and placed in a modified Krebs-Henseleit (Krebs') solution of the following composition (mM): NaCl, 115.21; KCl, 4.70; $CaCl_2$, 1.80; $MgSO_4$, 1.16; KH_2PO_4, 1.18; NaH CO_3, 22.14; dextrose, 7.88. Disodium ethylenediamine tetracetic acid (EDTA 0.03 mM) was added to suppress oxidation of amines. The Krebs' solution was oxygenated with a mixture of oxygen-carbon dioxide (95:5). Tissues not used immediately were stored for up to 24 hours at 4°C in Krebs' solution continuously oxygenated with 95% O_2 - 5% CO_2. No changes in tissue responsiveness have been observed during storage. Helically-cut strips were prepared according to methods previously described (Dyer,

1970). Histological examination of strips at the completion of an experiment indicated that greater than 80% of the endothelial cells remained intact. Furthermore, rubbing the strips did not change the responsiveness to 5-HT. The tissues were placed under 1 g of tension in 10-ml isolated organ baths maintained at 37°C and contractions were recorded isotonically. The tissues were allowed to equilibrate for at least 90 minutes before beginning the experiment. Concentration-response data were obtained by cumulative additions of the agonists in approximately one-half log increments (van Rossum, 1963).

Determination of agonist potencies

Concentration-response data to 5-HT and other serotonergic agonists were obtained after each tissue had been exposed to KCl (150 mM) and this response to KCl was set as the 100% response. EC_{50} values for agonists in the experiment were recorded at the molar concentration where the curves intersected the 50% level of the response axis to that agonist. Relative potencies were calculated by comparing the concentration of agonist required to produce a contraction equivalent to that of 5-HT at the 30% response. No calculations were possible for TFMPP and 2-MPP since their maximum response did not reach the 30% level in this study.

Determination of agonist dissociation constants (K_A) and relative efficacies (e_r)

Matched strips from an adjoining portion of the same uterine artery were used in all experiments to determine the K_A and e_r values for 5-HT (the reference agonist) or DOM. A concentration-response relationship to each agonist was obtained in a separate tissue prior to exposing the

tissue to dibenamine in order to inactivate a fraction of the receptors. To avoid the possibility of desensitization, the highest concentration of agonist added was that which would produce about 90% of the maximal response to that agonist. After fractional inactivation of the receptors by dibenamine ($2.5\text{-}7.5 \times 10^{-8}$M for 15 minutes), the bath fluid was changed 5 or 6 times over 30 minutes. The concentration-response relationship for that agonist was then repeated. The concentration of dibenamine used in the present study usually reduced the maximal response to an agonist by 20 to 40%, a reduction suitable for K_A determinations.

K_A values were determined as described by Furchgott and Bursztyn (1967). The reciprocals of the concentrations of an agonist before dibenamine treatment (1/[A]) were plotted against the reciprocals of the corresponding equieffective concentrations after treatment (1/[A']). From the slope and intercept of the straight line fitting the points, the values for K_A and for the fraction of active receptors remaining (q) were calculated on the basis of the equation (Furchgott, 1966):

$$\frac{1}{[A]} = \frac{1-q}{q\,K_A} + \frac{1}{q[A']} \qquad (1)$$

according to which K_A equals (slope-1)/intercept, and q equals 1/slope. In each experiment, the relative affinity for DOM as compared to 5-HT was obtained by dividing the K_A value obtained for 5-HT by that obtained for DOM.

The K_A values for 5-HT and that for DOM determined in an experiment on paired strips were then used to calculate the respective fractional

occupation of receptors by each agonist for each concentration used in establishing the control concentration-response curves prior to dibenamine treatment. The fractional occupancy was calculated from the equation (Furchgott and Bursztyn, 1967):

$$\frac{[RA]}{[R_T]} = \frac{[A]}{[A] + K_A} \qquad (2)$$

Where [RA] is the concentration of the receptor-agonist complex and $[R_T]$ is the total concentration of receptors. Control pre-dibenamine response data for 5-HT and DOM were then replotted to show response as a function of log $[RA]/[R_T]$. The relative efficacy (e_r) of DOM compared to 5-HT was the antilogarithm of the mean distance between the two agonists' curves along the abscissa.

In all experiments, cocaine (3×10^{-6}M) was added to block uptake mechanisms (Dyer, 1970), phentolamine (10^{-7}M) to inhibit alpha adrenergic receptors and iproniazid (0.36 mM) to block monoamine oxidase (MAO). Iproniazid was added for 40 minutes and the tissues were then washed 4 times over 30 minutes with fresh Krebs' solution. Cocaine and phentolamine were added 15 minutes prior to adding the agonists.

Determination of apparent dissociation constants for antagonists

Methiothepin, ketanserin and MDL 72222 were used in a series of experiments to determine the subtype of 5-HT receptors involved in contraction of the ovine uterine artery. Initially a concentration response relationship was obtained to a specific agonist. Then antagonists were allowed to equilibrate for 1 hour with the tissue before repeating the concentration response relationship to the same

agonist. The displacement of the log concentration-response curve for the agonist in the presence of the antagonist from that of the control was determined.

The time-related shift of the agonist's response curve was measured in matched preparation not treated with antagonist. The concentration ratio (CR_T) (EC_{50} at time t/EC_{50} at time 0) was determined from the two control agonists' concentration-response curves. The concentration ratio (CR) (EC_{50} in the presence of antagonists/EC_{50} in the absence of antagonists) obtained for the antagonist treated tissue was then adjusted according to the following formula:

$$\text{Adjusted CR} = \frac{CR}{CR_T}$$

Cocaine, phentolamine and iproniazid were also used as described above.

To quantify antagonism, the method described by Schild (1947) for the determination of pA_2 values was employed. The adjusted concentration ratio (see above) obtained from the shifts in the agonist concentration response curve by the different concentrations of the antagonist were utilized in the Schild equation (Arunlakshana and Schild, 1959)

$$\log (CR-1) = \log [B] - \log K_B \qquad (3)$$

where [B] is the molar concentration of the antagonist and the CR is the adjusted concentration ratio. A linear regression performed on the line generated by plotting log (CR-1) vs. - log [B] will have a slope of -1 if blockade is competitive. The intercept along the abscissa (i.e., when CR = 2) represents the negative log of the dissociation constant

(K_B) for a competitive antagonist (i.e., pA_2). The mathematical calculations involved in obtaining a pA_2 value were performed using a computer program which is based on procedures outlined by Tallarida and Murray (1981).

Apparent dissociation constants (K_B) for ketanserin against 2-methyl-5-HT and 8-OH-DPAT and the phenylpiperazines against 5-HT were determined for each concentration of antagonist according to the following equation (Furchgott, 1972):

$$K_B = \frac{[B]}{CR - 1} \qquad (4)$$

Where [B] is the concentration of the antagonist and CR is the adjusted concentration ratio as discussed above.

Experiments to evaluate alpha-adrenergic receptor stimulation

Experiments were performed to evaluate the possibility that alpha adrenergic receptor stimulation was involved in the response to 5-HT. Pairs of adjacent strips from a single uterine artery were studied to compare the antagonism of prazosin and yohimbine to 5-HT and norepinephrine (NE). The strips were first exposed to KCl (150 mM) and then washed to permit relaxation to the original resting level. Prazosin or yohimbine were added to the bath and allowed to equilibrate with the tissue for 20 minutes. The preparations were then tested with a series of cumulative additions of 5-HT or NE in the presence of the antagonists. The data were plotted to determine the displacement of the log concentration-response curve for the agonist in the presence of the antagonists.

Drugs

The following drugs were used: cocaine HCl; serotonin creatinine sulfate (Sigma Chemical Co., St. Louis, MO); R(-)-2,5-dimethoxy-4-methyl-amphetamine (National Institute of Drug Abuse, Rockville, MD); 8-hydroxy-dipropylaminotetralin (8-OH-DPAT), α-methyl-5-hydroxytryptamine, 2-methyl-5-hydroxytryptamine, m-trifluoromethyl-phenylpiperazine HCl (TFMPP), 1-(3-chlorophenyl)piperazine HCl (m-CPP), 1-(2-methoxyphenyl) piperazine HCl (2-MPP), 3-tropanyl-3,5-dichlorobenzoate (MDL 72222) (Res. Biochem. Inc., Natick, Massachusetts); Ketanserin tartrate (Janssen, Beerse, Belgium); iproniazid, methiothepin maleate (Hoffmann-LaRoche, Nutley, NJ); prazosin HCl (Pfizer Inc., Brooklyn, NY); yohimbine HCl (Merck, Rahway, NJ); phentolamine methane sulfate (CIBA Pharmacentical Company, Summit, NJ); dibenamine HCl (Smith, Kline and French Lab., Philadelphia, PA); l-norepinephrine bitartrate (Calbiochem Behring Corp., La Jolla, CA). Drugs were dissolved in saline, except for dibenamine and MDL 72222, which were dissolved in alcohol and diluted in saline just prior to use.

Data were expressed as means ± S.E.; for each experiment, n refers to the number of sheep from which vessels were taken. The student's t-test was used for statistical analysis of the difference of means.

Results

Contractions of ovine uterine artery by 5-HT, DOM and other serotonergic agonists

Serotonin, α-methyl-5-HT and DOM were found to be potent agonists

of ovine uterine arteries from pregnancy. The contractions were smooth and readily reproducible. Typical concentrations required for threshold contractions were about 10^{-8}M. All other agonists required a higher concentration to produce a threshold response. Concentration-response curves to 5-HT, DOM and other agonists are illustrated in Figure 1. In Table 1 the EC_{50} values and potency ratios are presented. The potencies of the agonists were determined to be DOM > 5-HT $\geq$ α-methyl-5-HT >> 8-OH-DPAT > 2-methyl-5-HT > mCPP > TFMPP. DOM and α-methyl-5-HT were full agonists relative to 5-HT, their Emax values being no different from 100%. The maximal contractile effects elicited by the other agonists tested were significantly less than those obtained with 5-HT and DOM. 2-MPP caused minimal contraction of the uterine artery with the maximal response being 2 percent of that to 5-HT.

Agonist dissociation constants (K_A)

The response to 5-HT before and after exposing the tissue to dibenamine (7.5×10^{-8}M for 15 min) is shown in Figure 2. Dibenamine reduced the maximal response to 5-HT about 40%. Figure 3 illustrates a double reciprocal plot of equieffective concentrations of 5-HT before (1/A) and after (1/A') dibenamine treatment in a single experiment. The mean dissociation constant (K_A) for the seven tissues was $3.7 \pm 0.7 \times 10^{-7}$M (Table 2). Results of experiments concurrently carried out with DOM are presented in Table 2. The mean K_A value for DOM was $1.8 \pm 0.3 \times 10^{-7}$M. It should be noted that the estimated fraction of receptors remaining active (q value) after pretreatment with dibenamine was essentially the same for 5-HT (0.19 ± 0.09) and DOM (0.25 ± 0.04). The results of the experiments with DOM were also used to evaluate the

Fig. 1. Concentration-contractile response relationships for 5-HT and other serotonergic agonists. Results are illustrated as the $\bar{x} \pm$ S. E. of the tissues from 3 to 8 animals and is expressed as a percentage of the contraction obtained with 150mM KCl

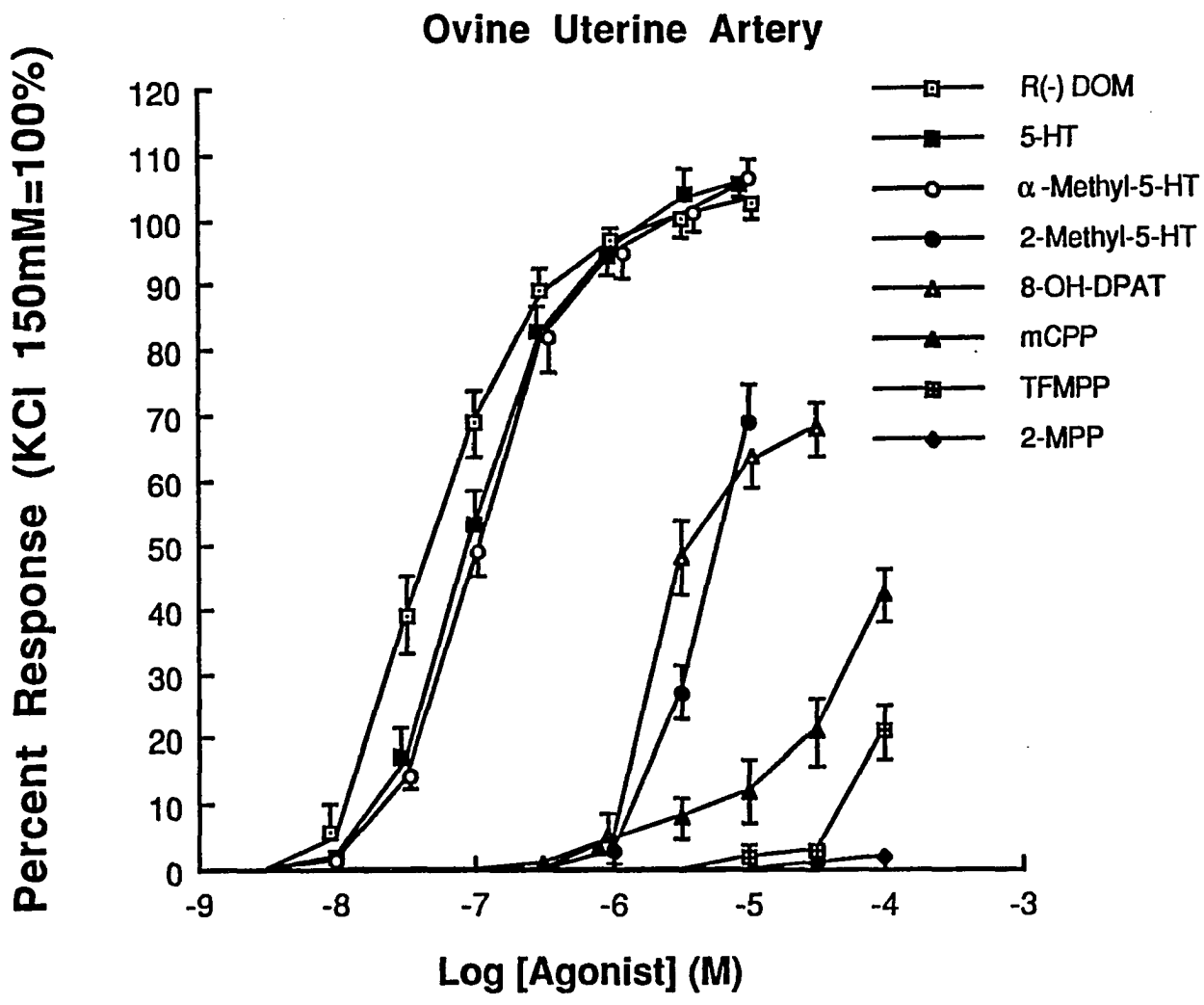

Ovine Uterine Artery
Percent Response (KCl 150mM=100%)
120
110
100
90
80
70
60
50
40
30
20
10
0
-9
-8
-7
-6
-5
-4
-3
Log [Agonist] (M)
R(-) DOM
5-HT
α -Methyl-5-HT
2-Methyl-5-HT
8-OH-DPAT
mCPP
TFMPP
2-MPP

Table 1. Comparative properties of serotonergic agonists on the ovine uterine artery

Agonists	N[a]	EC_{50}[b]	Relative Potency[c]	Approximate EC_{100}[d]	Percentage of Maximum Tissue Response Obtained at EC_{100}[e]
		M		M	%
5-HT	8	1.27×10^{-7}	1.000	1.0×10^{-5}	100
DOM	8	5.41×10^{-8}	2.000[f]	1.0×10^{-5}	98
α-methyl-5-HT	6	1.34×10^{-7}	0.934	1.0×10^{-5}	100
2-methyl-5-HT	4	3.88×10^{-6}	0.014[f]	1.0×10^{-5}	66
8-OH-DPAT	4	2.14×10^{-6}	0.025[f]	3.0×10^{-5}	65
mCPP	3	3.21×10^{-5}	0.001[f]	1.0×10^{-4}	40
TFMPP	3	5.35×10^{-5}		1.0×10^{-4}	20
2-MPP	3	3.30×10^{-5}		1.0×10^{-4}	2

[a]N is the number of animals.

[b]EC_{50} is the effective concentration to produce 50% of the maximal response to the respective agonist.

[c]Relative Potency was calculated at the EC_{30} (See Methods), 5-HT arbitrarily set at 1. Values for TFMPP and 2-MPP were not calculated since contraction to these agonists did not reach the EC_{30}.

[d]EC_{100} is the effective concentration to produce 100% of the maximal response to the respective agonist.

[e]5-HT is arbitrarily set at 100%.

[f]Significantly different from 1 ($P<0.05$).

Fig. 2. Effect of treatment with dibenamine on 5-HT-elicited contractions of the isolated ovine uterine artery in late pregnancy. Contractions to 5-HT were obtained before and after exposure to 7.5×10^{-8}M dibenamine for 15 min. Dibenamine was washed out of the tissue before obtaining the second concentration-response relationship to 5-HT. The left panel represents the response of the paired tissue (strip 1) to 5-HT over the course of the experiment (time control)

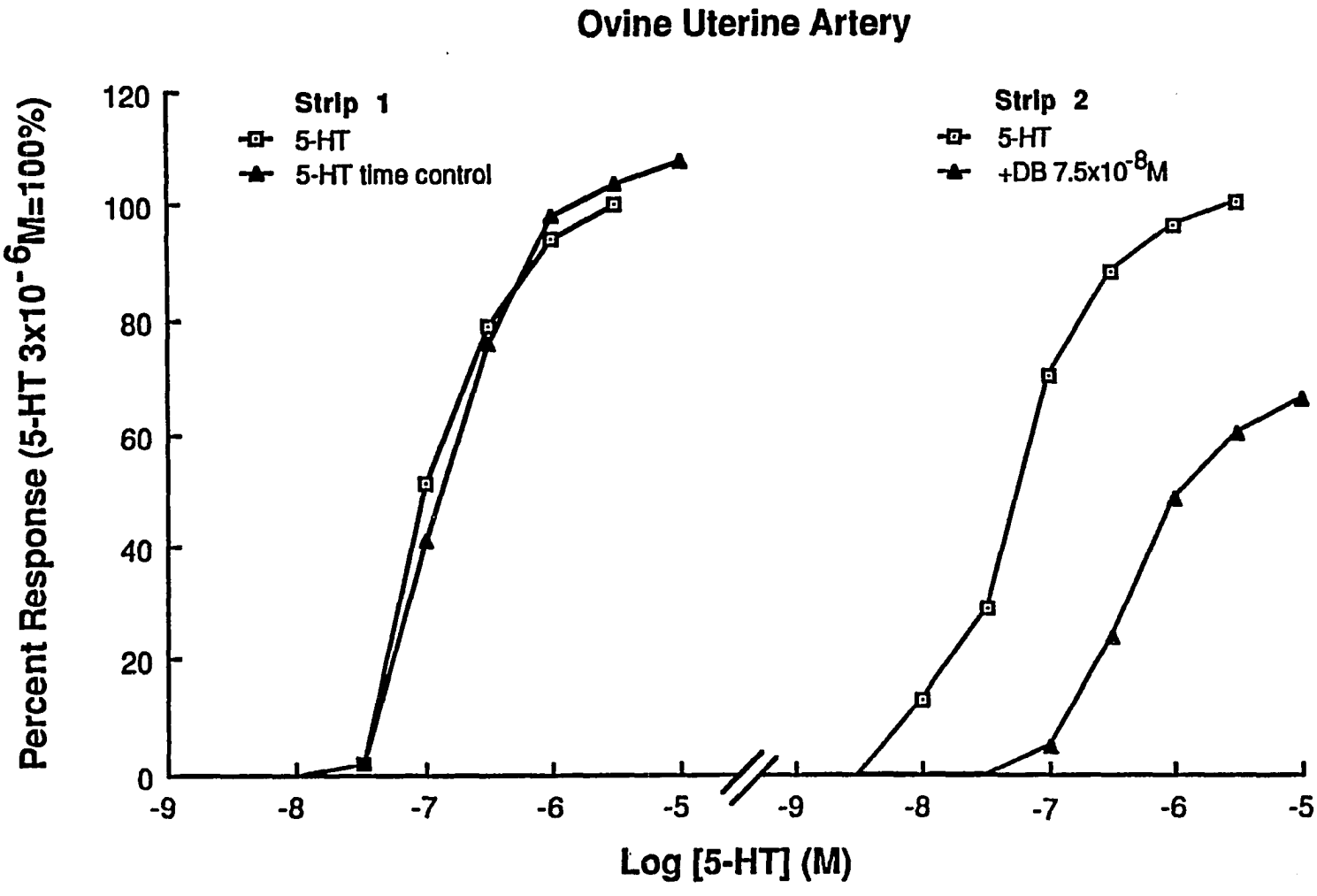

Ovine Uterine Artery
Strip 1
5-HT
5-HT time control
Strip 2
5-HT
+DB 7.5x10-8M
Percent Response (5-HT 3x10-6M=100%)
120
100
80
60
40
20
0
-9
-8
-7
-6
-5
-9
-8
-7
-6
-5
Log [5-HT] (M)

Fig. 3. Determination of the dissociation constant for 5-HT acting on serotonergic receptors using the tissue responses illustrated in Figure 2. A double reciprocal plot of equiactive concentrations before and after incubation with dibenamine were used to determine the dissociation constant (K_A) of 5-HT and the fraction of receptors still active (q) after dibenamine (see "Methods")

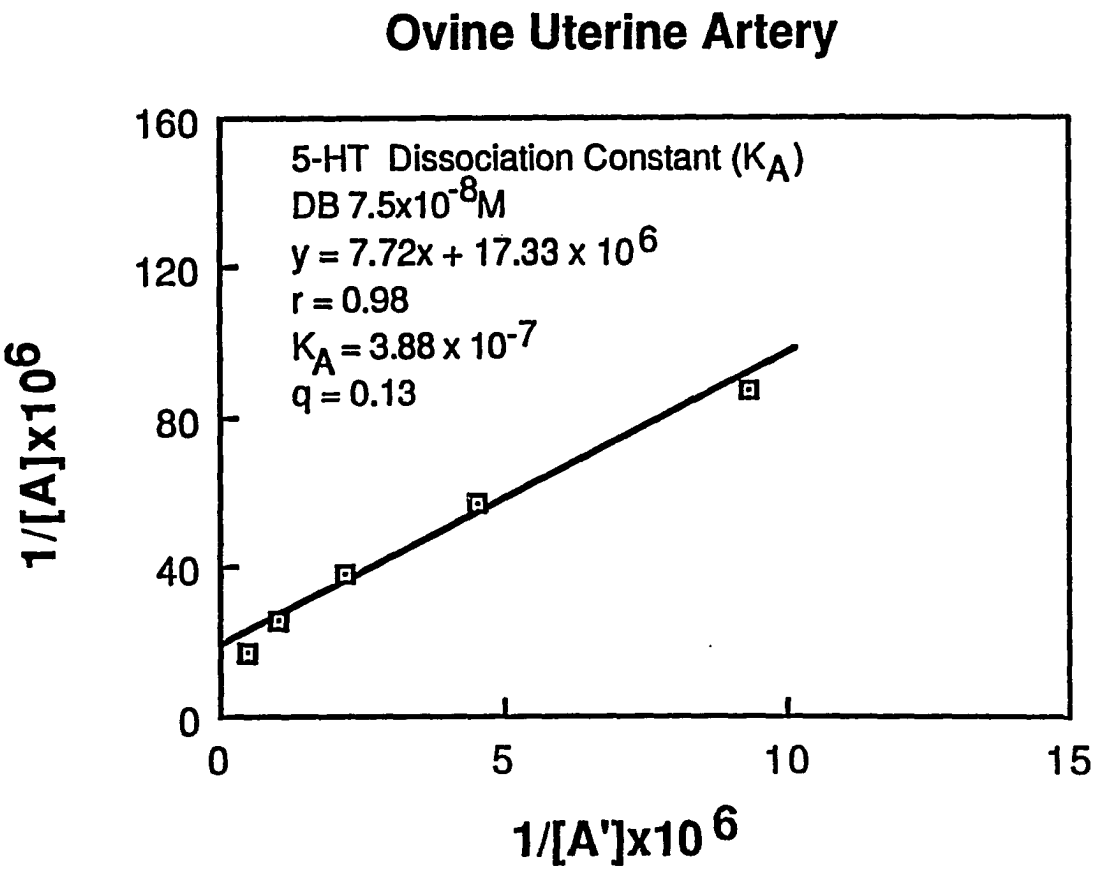

Ovine Uterine Artery
5-HT Dissociation Constant (K_A)
DB $7.5x10^{-8}$M
$y = 7.72x + 17.33 \times 10^6$
$r = 0.98$
$K_A = 3.88 \times 10^{-7}$
$q = 0.13$
160
120
80
40
0
0
5
10
15
1/[A]x10^6
1/[A']x10^6

Table 2. Comparison of dissociation constants (K_A), relative efficacy (e_r), affinity and fraction of receptors remaining active after dibenamine (7.5×10^{-8}M) treatment (q) for 5-HT and DOM acting on the serotonergic receptors of ovine uterine artery

Agonists	N[a]	K_A	q	e_r[b]	Relative Affinity[c]
		M			
5-HT	7	$3.7 \pm 0.7 \times 10^{-7}$	0.19 ± 0.09	1.00	1.00
DOM	7	$1.8 \pm 0.3 \times 10^{-7}$	0.25 ± 0.04	1.18 ± 0.17	1.80 ± 0.24[d]

[a]N is the number of animals.
[b,c]5-HT is arbitrarily set at 1.
[d]Significantly different from 1 ($P<0.05$).

relationship between the estimated K_A for DOM and the q values after treatment with two different concentrations of dibenamine. The value for q declined from 0.45 to 0.25 as the concentration of dibenamine was increased from 2.5×10^{-8}M to 7.5×10^{-8}M. Despite this significant decline in q, the estimated mean K_A values of DOM remained essentially the same.

In each experiment with paired strips, the affinity of DOM relative to that of 5-HT was calculated by dividing the estimated K_A of 5-HT by that of DOM. The affinity of DOM was on the average about 80% greater than that of 5-HT ($P < 0.05$) (Table 2).

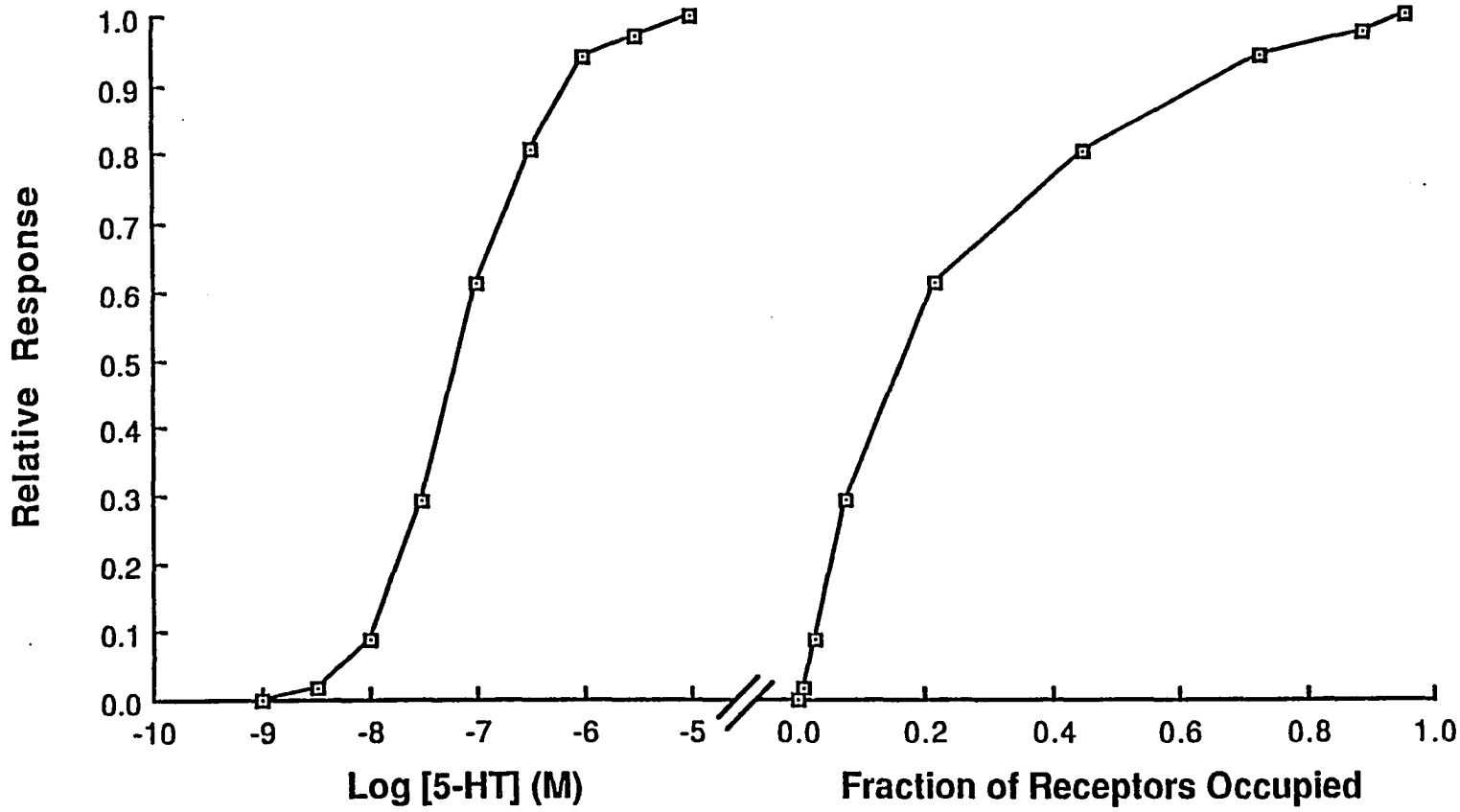
Relative efficacy (e_r) of DOM

The individual concentration-response curves to DOM and 5-HT that were obtained in the above experiments were compared. The concentration-response curves before exposure to dibenamine were replotted as response against log RA/R_T which was determined from the relevant K_A value (see "Methods"). The relative efficacy of DOM to 5-HT was obtained from individual experiments as the distance along the log RA/R_T axis. The mean values (± S.E.) are presented in Table 2. The efficacy of DOM was not significantly different from that of 5-HT.

Relation of response to the fraction of receptors occupied by 5-HT and DOM

The average K_A value for 5-HT (3.7×10^{-7}M) was used in Equation 2 to calculate the fraction of receptors occupied ($[RA]/[R_T]$) at each concentration employed in obtaining the complete concentration-response curve shown in Figure 4 (left panel). The result of replotting relative response against the calculated values for fractional receptor

Fig. 4. Relationship of the relative response to the log concentration and to the fraction of 5-HT receptors occupied. Left panel: Log concentration-response curve drawn through average data points obtained on the strips from 21 animals (control strips in experiments to determine the pA_2 of ketanserin and methiothepin as antagonists to 5-HT and control tissues in determining the K_A for 5-HT). The contractile response at each concentration is shown relative to that obtained at 5-HT (3×10^{-6}M). Right panel: Replot of data from the left panel showing the relative response as a function of the fraction of receptors occupied by 5-HT. The fraction of receptors occupied at each concentration was calculated from equation 2, employing the average K_A value for 5-HT of 3.7×10^{-7}M


Relative Response
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-10
-9
-8
-7
-6
-5
Log [5-HT] (M)
0.0
0.2
0.4
0.6
0.8
1.0
Fraction of Receptors Occupied

occupation is shown in Figure 4 (right panel). The curves indicate that one-half the maximal response is obtained when only about 17% of receptors are occupied by 5-HT, and that 90% of the maximum is obtained when about 65% of receptors are occupied. Similar results were found with DOM in that 22% of the receptors are occupied at one-half the maximal response and about 54% of the receptors are occupied at 90% of the maximum response.

Studies using competitive inhibitors

Three antagonists of 5-HT receptors were used in this investigation. These included: methiothepin, ketanserin and MDL 72222. Ketanserin competitively inhibited responses to 5-HT as demonstrated by parallel shifts in the log concentration-response curves to the right (Figure 5). Maximal responses were not affected by ketanserin at the highest concentration used (10^{-7}M). The curves for DOM were shifted to the right by ketanserin about the same as those for 5-HT. Schild plots for ketanserin vs. 5-HT and DOM yield straight lines with the slopes not significantly different from unity. The pA_2 values for ketanserin vs. 5-HT and DOM are shown in Figure 6. Ketanserin (10^{-8}M) effectively blocked the contractions to 8-OH-DPAT and 2-methyl-5-HT and shifted their concentration-response curves in a parallel manner to the right without affecting the maximum response. The dissociation constants (K_B) of ketanserin vs. 8-OH-DPAT and 2-methyl-5-HT were 2.49 ± 0.71 nM (n=3) and 2.88 ± 0.59 nM (n=3), respectively.

Four different concentrations ranging from 6.4×10^{-10}M to 2.1×10^{-8}M of methiothepin were employed to test for antagonism of contractions to 5-HT. The concentration-response curves for 5-HT were shifted to the

Fig. 5. Cumulative concentration-response curves for 5-HT obtained with isolated ovine uterine artery in late pregnancy in the presence of various concentrations of ketanserin after equilibration for 60 min. Each point represents the $\bar{x} \pm$ S.E. of the tissues from 8 animals and is expressed as a percentage of the contraction obtained to 3×10^{-6}M 5-HT in control experiments

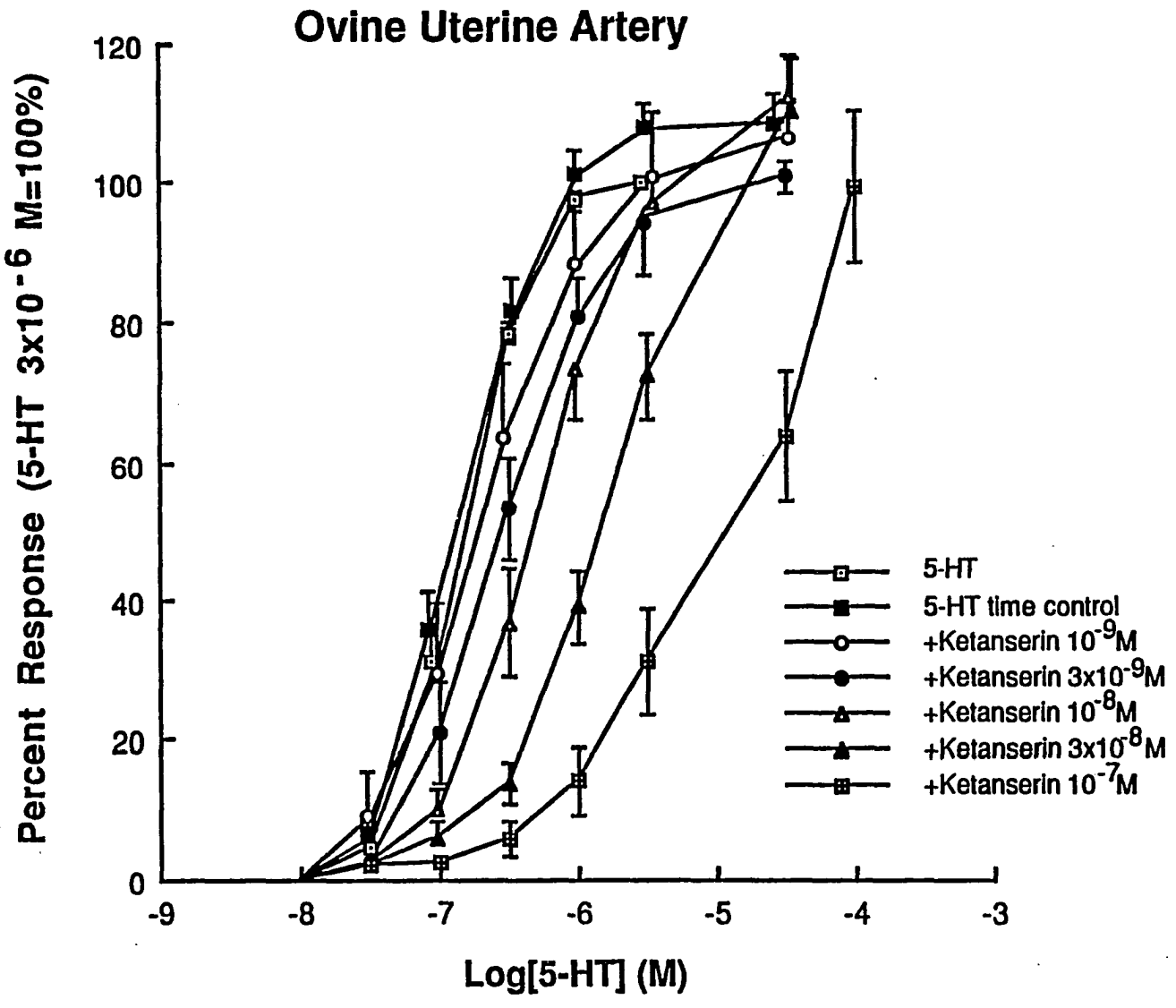
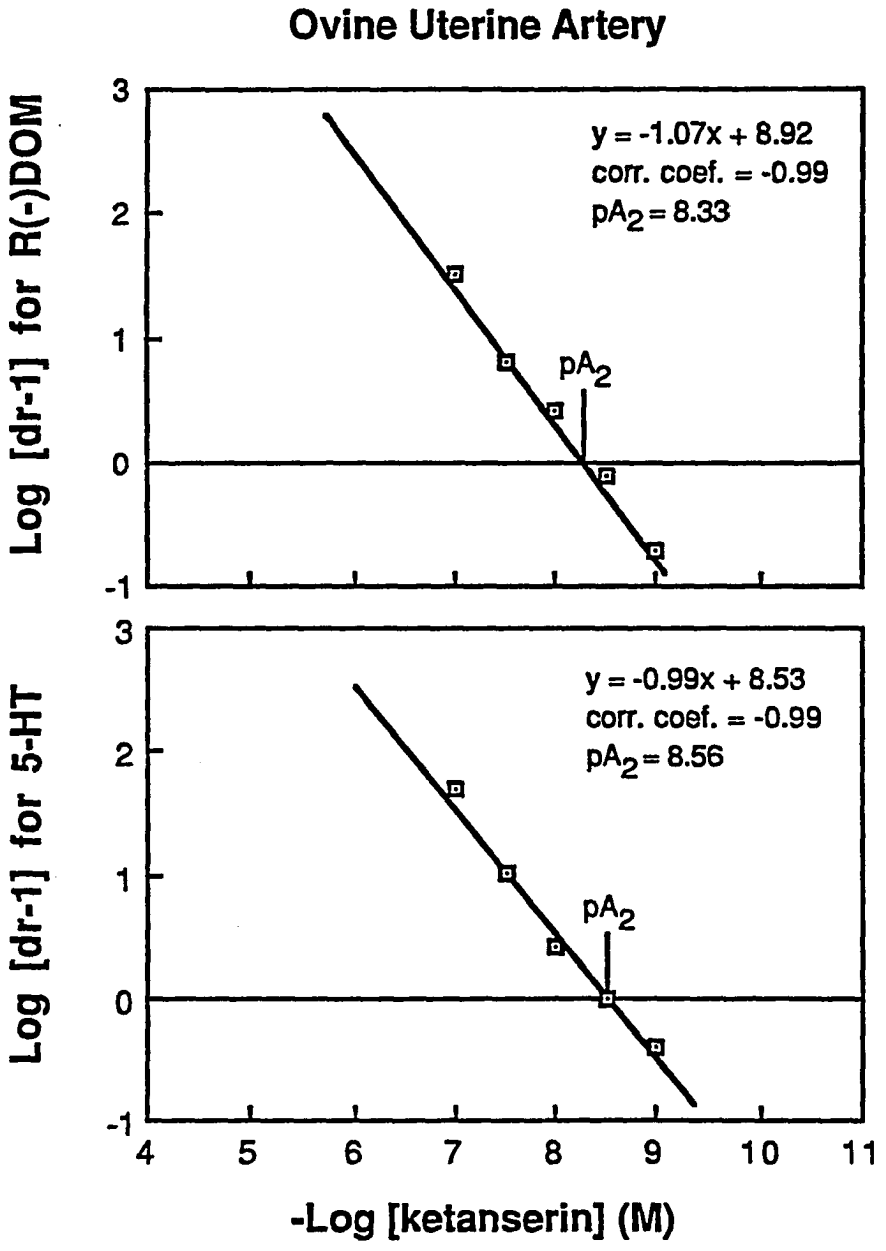

Ovine Uterine Artery
Percent Response (5-HT 3x10-6 M=100%)
120
100
80
60
40
20
0
-9
-8
-7
-6
-5
-4
-3
Log[5-HT] (M)
5-HT
5-HT time control
+Ketanserin 10-9M
+Ketanserin 3x10-9M
+Ketanserin 10-8M
+Ketanserin 3x10-8M
+Ketanserin 10-7M

Fig. 6. Schild plot for determination of the pA_2 for ketanserin when tested against 5-HT (bottom) and DOM (above) on isolated ovine uterine artery in late pregnancy. Each point represents the average of the experiments from 8 animals. The intercept on the abscissa gives the pA_2 value. The slopes of the fitted regression lines are shown in the figure


Ovine Uterine Artery
Log [dr-1] for R(-)DOM
y = -1.07x + 8.92
corr. coef. = -0.99
pA2 = 8.33
pA2
Log [dr-1] for 5-HT
y = -0.99x + 8.53
corr. coef. = -0.99
pA2 = 8.56
pA2
3
2
1
0
-1
4
5
6
7
8
9
10
11
-Log [ketanserin] (M)

right by methiothepin with depression of the maximal response. At the highest concentration of methiothepin used (2.1×10^{-8}M), the maximal response for 5-HT was depressed to 68 ± 4.9% (Figure 7). The Schild plot for methiothepin vs 5-HT yields a straight line with the slope being -1.27 which was significantly different from unity. The pA_2 value determined by the Schild plot was 8.94.

Figure 8 presents the results of the antagonism by MDL 72222 of responses to 5-HT. MDL 72222 (10^{-8} to 10^{-6}M) had little or no effect on contractions to 5-HT. MDL 72222 (10^{-8}M) shifted the concentration-response curve for 5-HT slightly to the left and decreased the EC_{50} 1.7 fold which was not significantly different from the control. MDL 72222 (10^{-6}M) had no effect on the contractile responses to 2-methyl-5-HT.

Antagonist activities of the phenylpiperazine derivatives on 5-HT-induced contractions

Figure 9 presents the results of the antagonism of responses to 5-HT by 2-MPP, TFMPP and mCPP. No agonist activity of 2-MPP (up to 10^{-4}M) occurred in this smooth muscle preparation. TFMPP (10^{-5}) and mCPP (10^{-5}) produced 5% and 11%, respectively, of the response to 5-HT (Fig. 9). All three phenylpiperazines effectively blocked the contractile responses to 5-HT and shifted the concentration-responses curves to the right in a parallel manner without changes in the maximum response (Fig. 9). Among the three phenylpiperazines, mCPP was the most potent antagonist against 5-HT-induced contractions, followed by TFMPP and 2-MPP. The dissociation constants (K_B) of these phenylpiperazines against 5-HT-induced contraction were determined to be: mCPP, 0.13 ± 0.06 μM (n=3); TFMPP, 0.22 ± 0.08 μM (n=3); 2-MPP, 1.43 ± 0.54 μM (n=3).

Fig. 7. Cumulative concentration-response curves for 5-HT obtained with isolated ovine uterine artery in late pregnancy after equilibration for 60 min. in the presence of various concentrations of methiothepin. Each point represents the $\bar{x} \pm$ S.E. of the tissues from 7 animals and is expressed as a percentage of the contraction obtained to 3 x 10^{-6}M 5-HT in control experiments

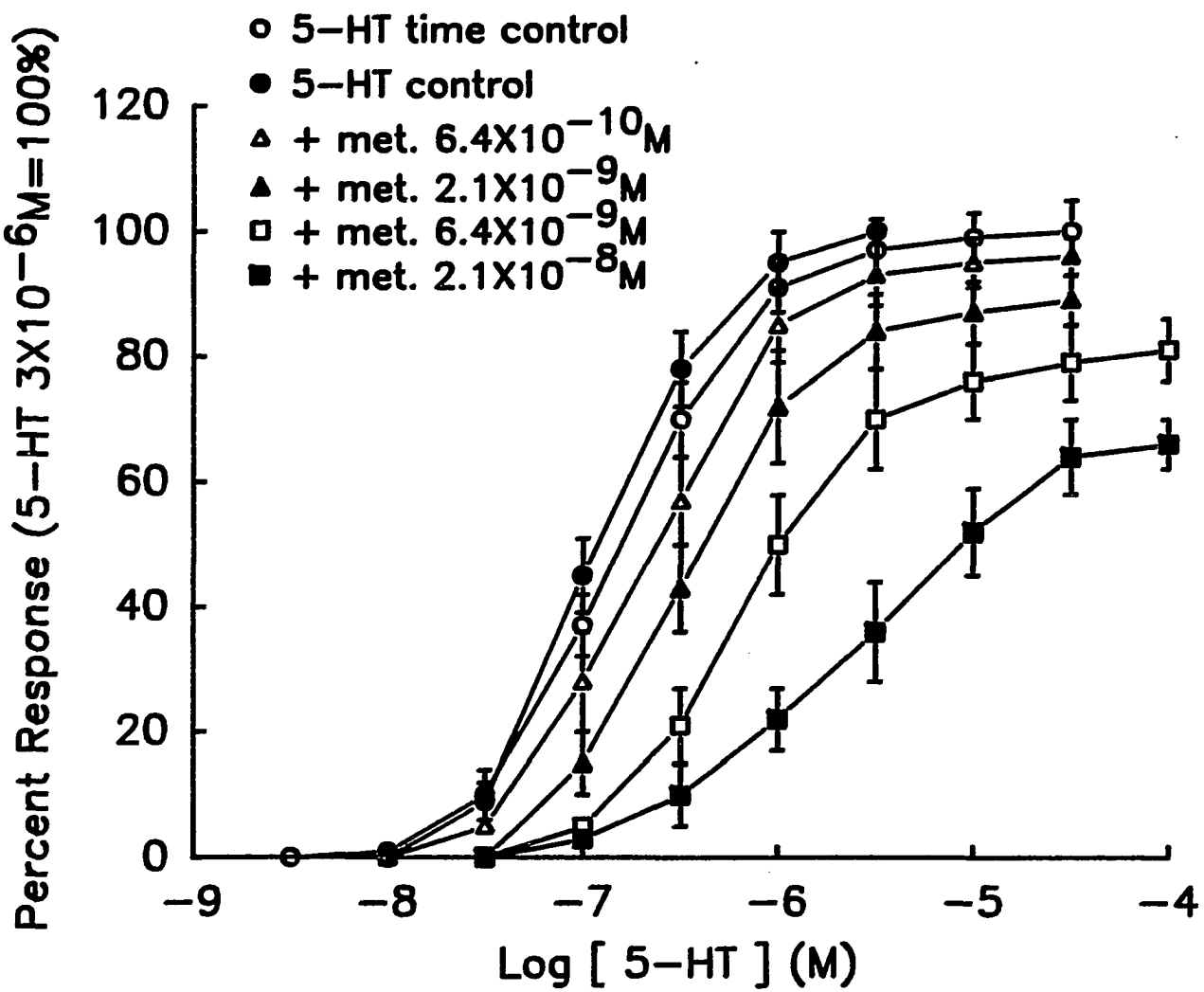

5–HT time control
5–HT control
+ met. 6.4X10^{-10}M
+ met. 2.1X10^{-9}M
+ met. 6.4X10^{-9}M
+ met. 2.1X10^{-8}M
Percent Response (5–HT 3X10^{-6}M=100%)
120
100
80
60
40
20
0
−9
−8
−7
−6
−5
−4
Log [5–HT] (M)

Fig. 8. Cumulative concentration-response curves for 5-HT obtained with isolated ovine uterine artery in late pregnancy after equilibration for 40 min. in the presence of various concentrations of MDL 72222. Each point represents the average of the tissues from 4 animals and is expressed as a percentage of the contraction obtained to 150mM KCl. *, significantly different from the control ($P<0.05$)

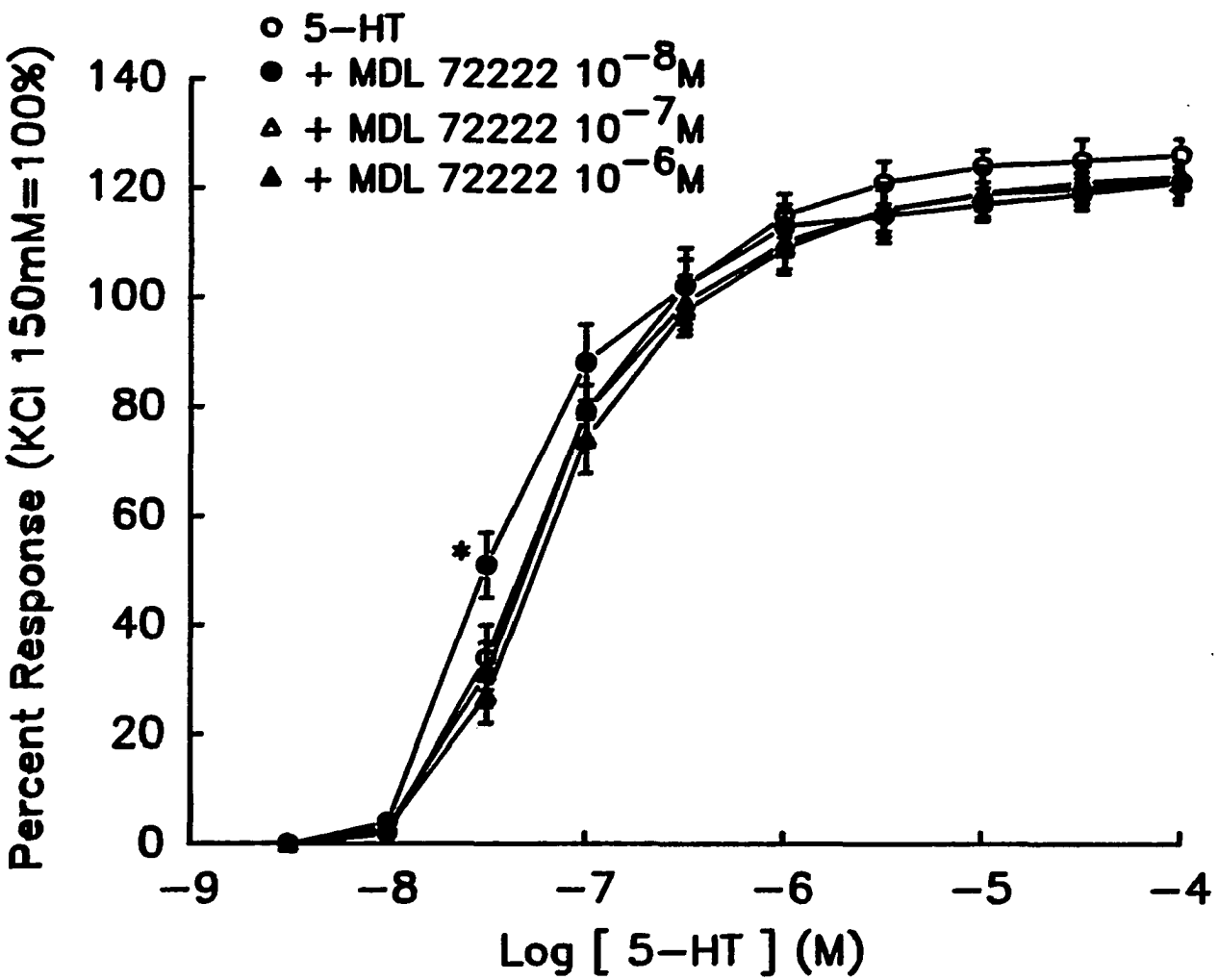
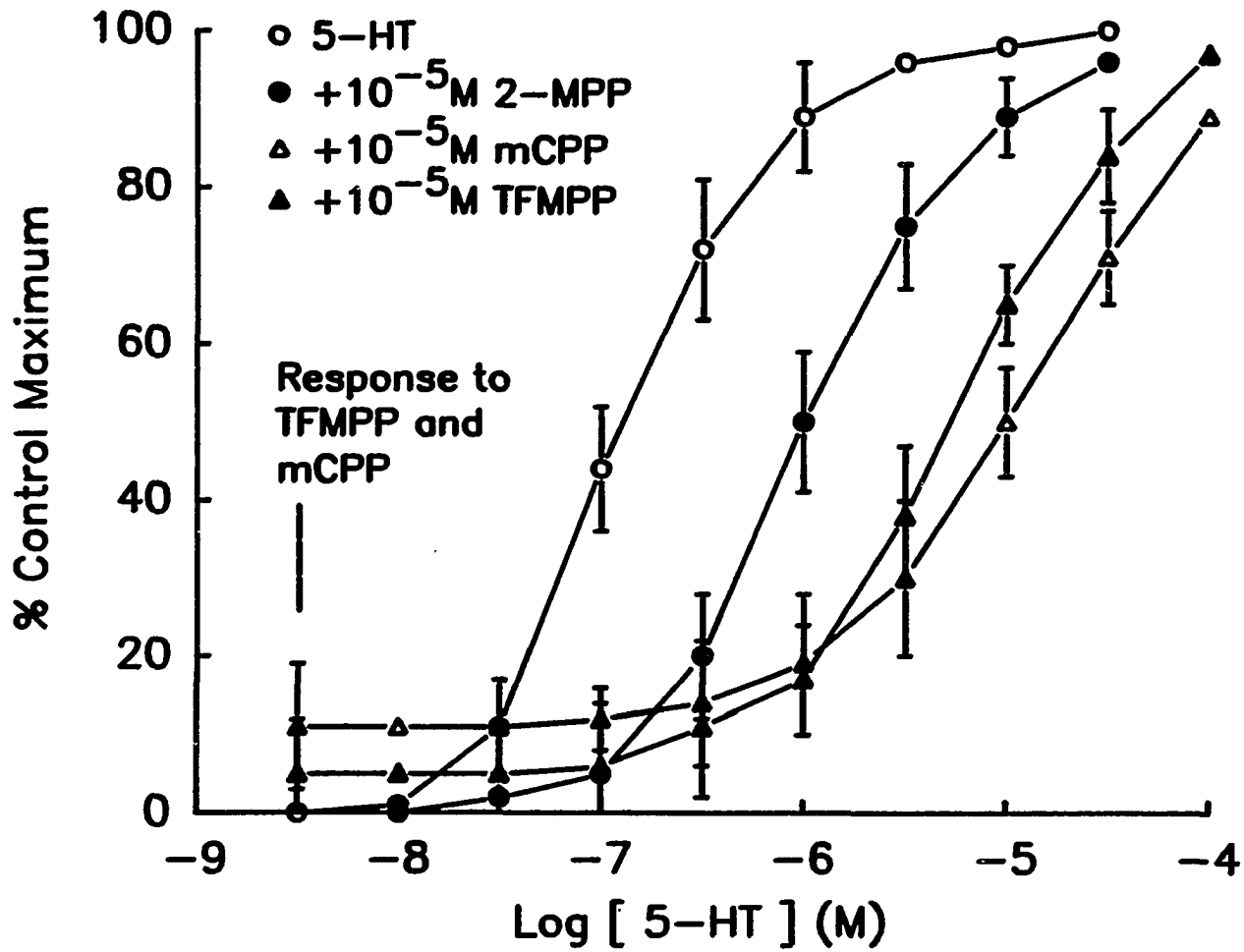

5–HT
+ MDL 72222 10⁻⁸M
+ MDL 72222 10⁻⁷M
+ MDL 72222 10⁻⁶M
Percent Response (KCl 150mM=100%)
140
120
100
80
60
40
20
0
−9
−8
−7
−6
−5
−4
Log [5–HT] (M)
*

Fig. 9. Cumulative concentration-response curves for 5-HT obtained with isolated ovine uterine artery in late pregnancy after equilibration for 40 min. in the presence of mCPP (10^{-5}M), TFMPP (10^{-5}M) and 2-MPP (10^{-5}M). 2-MPP lacked agonist activity. mCPP and TFMPP produced 11% and 5% contractions, respectively. Each point represents the $\bar{x} \pm$ S.E. of the tissues from 3 animals and is expressed as a percentage of the 5-HT control maximum


100
80
60
40
20
0
% Control Maximum
o 5–HT
● +10⁻⁵M 2–MPP
△ +10⁻⁵M mCPP
▲ +10⁻⁵M TFMPP
Response to
TFMPP and
mCPP
−9
−8
−7
−6
−5
−4
Log [5–HT] (M)

Activation of α-adrenergic receptors by 5-HT

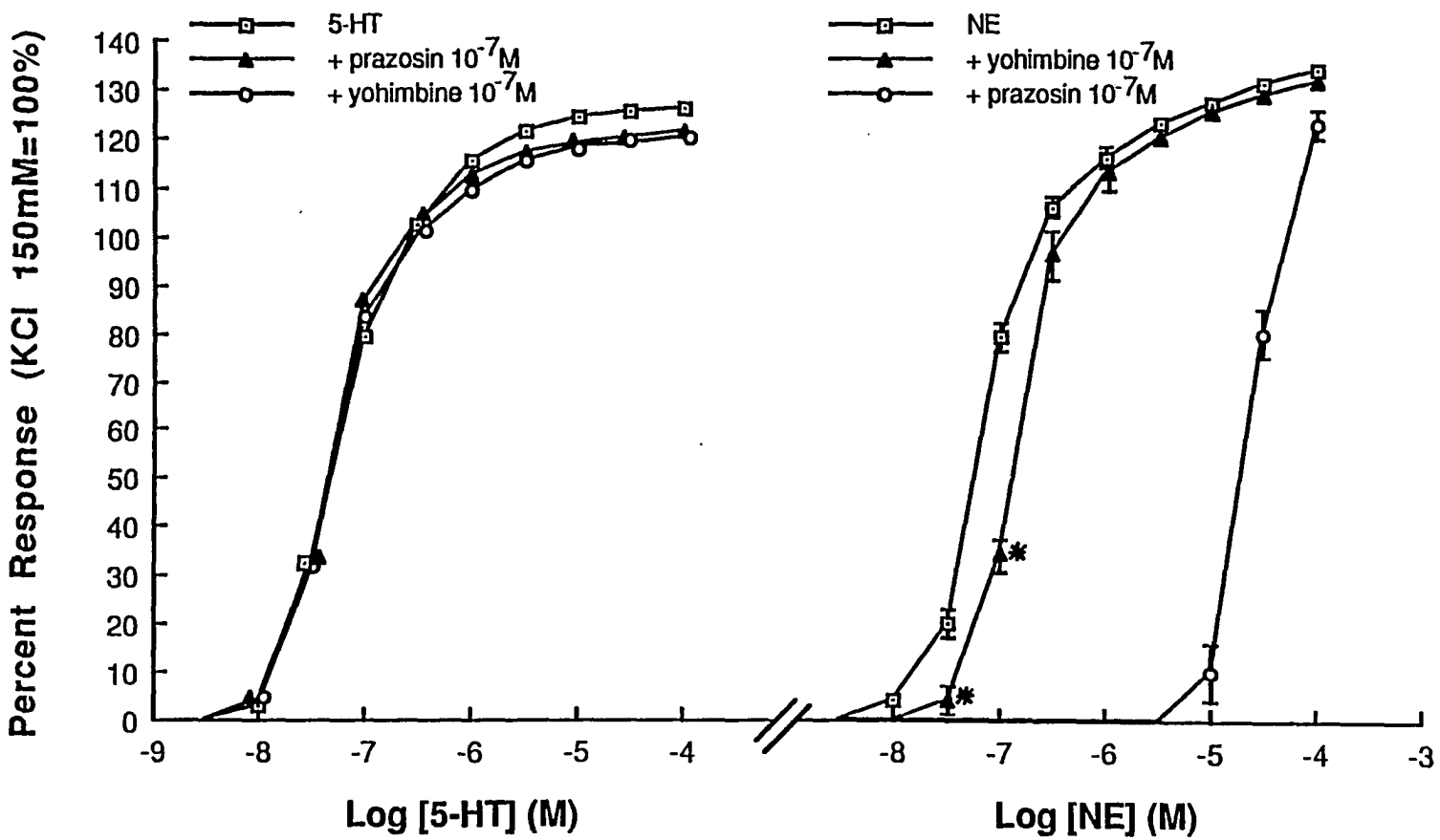
The ability of 5-HT to stimulate α-adrenergic receptors was evaluated in uterine artery strips pretreated with prazosin or yohimbine. Paired strips from the same artery were used to compare the antagonism of prazosin and yohimbine to NE. Figure 10 illustrates the results of this experiment. Both prazosin (10^{-7}M) and yohimbine (10^{-7}M) did not antagonize responses to 5-HT, but effectively antagonized those to NE. Yohimbine (10^{-7}M) increased the EC_{50} of NE 2.5 times ($P<0.05$), while prazosin (10^{-7}M) increased it 356 fold.

Discussion

The pA_2 values of ketanserin for 5-HT and DOM were not statistically different in this study (Figure 6). This finding provides strong evidence that both DOM and 5-HT evoke contractions of the late pregnant ovine uterine artery by acting on the same type of receptor. Additional evidence for this conclusion comes from the finding that when strips from the same artery were pretreated in an identical manner with dibenamine, the fraction of receptors remaining active (q) for DOM, was not significantly different from those remaining active for 5-HT (Table 2).

Ketanserin has been documented to be a potent and selective 5-HT_2 receptor antagonist (Leysen et al., 1981, Leysen et al., 1982; Van Nueten et al., 1981). In this study, ketanserin caused parallel shifts of the concentration-response curves for 5-HT and DOM to the right, consistent with competitive inhibition. This was supported by the

Fig. 10. Cumulative concentration-response curves to 5-HT (left panel) and norephinephrine (right panel) obtained with isolated ovine uterine artery strips in late pregnancy following equilibration with prazosin (10^{-7}M) or yohimbine (10^{-7}M) for 40 min. Each point represents the average of the tissues from 4 animals and is expressed as a percentage of the contraction obtained to 150mM KCl. *, significantly different from the control ($P<0.05$)


Percent Response (KCl 150mM=100%)
5-HT
+ prazosin 10-7M
+ yohimbine 10-7M
NE
+ yohimbine 10-7M
+ prazosin 10-7M
Log [5-HT] (M)
Log [NE] (M)
*
*

Schild plots whose slopes were not significantly different from unity. The pA_2 values of ketanserin against 5-HT (8.56) and DOM (8.33) in the present study are comparable to its affinity (Ki=2.1nM) for 5-HT_2 binding sites in brains (Leysen et al., 1981; Leysen et al., 1982) and its pA_2 values determined on peripheral blood vessels (8.1 to 9.7) by other authors (Cohen et al., 1983b; Cohen, 1986; Humphrey, 1984; Van Nueten et al., 1982, Van Nueten et al., 1981). This suggests that the contractile responses produced by 5-HT and DOM in the ovine uterine artery are mediated by 5-HT_2 receptors. The contractions produced by 8-OH-DPAT and 2-methyl-5-HT were also blocked by ketanserin with dissociation constants (2.49 nM and 2.88 nM, respectively) which were similar to those for 5-HT and DOM. This provides further evidence that 5-HT_2 receptors are present in the ovine uterine artery. The finding that DOM acts on 5-HT_2 receptors is in accord with our previous studies of ovine umbilical arteries (Dyer, 1983) and those reported by Glennon and co-workers in the central nervous system (1983, 1984).

The K_A values obtained for DOM after different degrees of receptor inactivation by dibenamine were not signficantly different. This suggests that 5-HT receptors remaining active after dibenamine treatment have the same characteristic (i.e., affinity) as the total population of original receptors prior to treatment. The K_A determined for DOM in the present study was about two times less than that for 5-HT (Table 2). The affinity of DOM was about 80% greater than that of 5-HT. Despite the difference in K_A values for DOM and 5-HT, they have essentially the same e_r (Table 2). If 5-HT is designated as a "full agonist" for the 5-HT receptor, then on the basis of its similar relative efficacy, DOM is

also a full agonist. This classification of DOM as a full agonist does not agree with those by Sanders-Bush and co-workers (1988) or of Dyer et al. (1973). Sanders-Bush et al. found DOM to be a partial agonist on the 5-HT_2 receptor which stimulates phosphoinositide hydrolysis in rat cerebral cortex. DOM stimulated phosphoinositide hydrolysis in the cerebral cortex with a maximum effect that was 76% of that produced by 5-HT. Dyer and co-workers found that DOM contracted the ovine umbilical artery 82% of that to 5-HT. The difference between this study and that of Sanders-Bush et al. or Dyer et al. could be from the different methodologies and/or tissues used to study DOM's action on the 5-HT receptor.

Phentolamine was used in the present studies to block activation of adrenergic receptors by 5-HT as reported by Innes (1962). In preliminary experiments we established that the K_B of phentolamine was 1.08×10^{-6}M when 5-HT was the agonist (data not presented). This is about 100-fold higher than the K_B value for phentolamine when phenylephrine was the agonist on strips of rabbit aorta (Furchgott, 1972). Since a concentration of phentolamine (10^{-7}M) was used to block adrenergic receptors in this study and this concentration is about ten-fold lower than the K_B when 5-HT is the agonist it is doubtful that phentolamine contributed to the antagonism of 5-HT or DOM by ketanserin or methiothepin.

Additional experiments were carried out to help ascertain whether or not 5-HT might be acting in part via alpha adrenergic receptors in the ovine uterine artery. Prazosin in a concentration (100 nM) which is approximately 30 times its reported K_B value of 3.5 nM in the rabbit

aorta (Furchgott, 1980) did not antagonize contractions to 5-HT but did increase the norepinephrine EC_{50} 356 fold (Figure 9). Similarly, yohimbine (10^{-7}M) had no effect on the response to 5-HT but increased the EC_{50} for norepinephrine 2.5 fold which was significantly from the control. The results clearly indicate that contractions to 5-HT in the ovine uterine artery are not mediated by adrenergic receptors. This conclusion is in agreement with those of Stollak and Furchgott (1984) and Apperley et al. (1976) based on their determination of the pA_2 values for a series of competitive antagonists in the rabbit aorta.

Theoretically, the same fractional occupation of receptors by either 5-HT or DOM in an individual artery strip would give the same degree of contraction of the strip. The relation between occupation of receptors and response in percentage of maximum for 5-HT and DOM were very similar in the present studies. Both agonists gave half of the maximum response with about 20% occupation, and 90% of the maximum with about 60% occupation. As predicted from inactivation studies with dibenamine, there is very little, if any, receptor reserve, since almost all the receptors need to be occupied to produce a maximal response. Evidence suggesting a lack of spare receptors for $5\text{-}HT_2$ receptors in other tissues was also obtained by Cohen et al. (1986a) in the rat jugular vein and uterus.

The relative potencies of various agonists acting on serotonergic receptors were determined by comparing the concentrations of each required to produce the 30 percent of maximum response. Differences in relative potencies among the agonists may result from differences in their affinities and/or their relative efficacies. In the case of DOM

and 5-HT which have equal relative efficacy, the difference in the relative potency can be attributed to the difference in their affinity for the 5-HT_2 receptor in the ovine uterine artery.

5-HT_{1A} receptor has been reported to be linked to contractions in the canine basilar artery (Peroutka et al., 1986; Taylor et al., 1986). However, in this study contractions to 8-OH-DPAT were effectively blocked by ketanserin, suggesting that 5-HT_{1A} subtype of receptors are not present in this tissue. The three phenylpiperazine derivatives used in this study, TFMPP, mCPP and 2-MPP, have been identified as relatively selective ligands for the 5-HT_{1B} binding sites in the rat brain (Sills et al., 1984; Fuller et al., 1980). However, these compounds can bind to 5-HT_2 binding sites as well. TFMPP and mCPP have only 3- to 18-fold selectivity for 5-HT_1 vs. 5-HT_2 sites (Glennon, 1987; Martin and Sanders-Bush, 1982). 2-MPP possesses a 100-fold selectivity for 5-HT_1 vs. 5-HT_2 sites and its affinity for 5-HT_1 sites is comparable to that of TFMPP (Glennon, 1987). These compounds are generally thought to be agonists at central 5-HT receptors with which they interact and which activate presynaptic autoreceptors on serotonin neurons that modulate the neurogenic release of serotonin (Markin and Sanders-Bush, 1982). However, in the present study, these compounds lacked agonist activity or had very weak agonist activity but instead antagonized the contractile responses produced by serotonin. As discussed above, the 5-HT-induced contraction was mediated by 5-HT_2 receptors in this tissue. It is apparent that these phenylpiperazine derivatives are better antagonists than agonists at 5-HT_2 receptors in the ovine uterine artery. Our results are totally consistent with those reported by Cohen

et al. (1983a, 1983b, 1986b). These authors demonstrated that mCPP and TFMPP were potent competitive antagonists at 5-HT_2 receptors in the rat jugular vein and aorta. The dissociation constants (K_B) of mCPP (0.13 μM) and TFMPP (0.22 μM) vs. 5-HT in this study were comparable to those (0.04 μM and 0.06 μM, respectively) reported by Cohen and Fuller (1983). The higher dissociation constant of 2-MPP (1.43 μM) than that of mCPP or TFMPP may be due to its lesser affinity at 5-HT_2 binding site than the other two phenylpiporazines, as discussed above. More recently, Sanders-Bush and Conn (1986) found that TFMPP and mCPP acted as pure antagonists of the central 5-HT_2 receptors. It has been demonstrated that TFMPP and mCPP failed to stimulate phosphoinositide hydrolysis at concentrations which completely inhibited the response to 5-HT in the rat cerebral cortical slices (Sanders-Bush and Conn, 1986). Therefore, the central agonists' activities of these phenylpiperazines may be mediated by different subtypes of 5-HT receptors.

Methiothepin is a nonselective antagonist with affinity for both 5-HT_1 and 5-HT_2 binding sites in the brain (Martin and Sanders-Bush, 1982; Engel et al., 1983). It has been demonstrated that methiothepin competitively antagonized the 5-HT-induced contraction in canine coronary and femoral arteries (Cohen, 1986; Houston and Vanhoutte, 1988). In the present study, methiothepin produced a potent concentration-dependent inhibition of 5-HT-induced contractions and shifted the concentration-response curves to the right in a parallel manner. However, the maximum response was depressed as well. The slope of the Schild plot was greater than one. This might be due to its noncompetitive nature of antagonism as reported in the canine saphenous

vein (Apperley and Humphrey, 1986) and in the endothelial responses of the canine coronary artery to 5-HT (Houston and Vanhoutte, 1988). The pA_2 value (8.94) determined in this study was similar to those reported in the canine femoral artery (8.8) by Cohen (1986) and in the canine coronary artery (8.78) by Houston and Vanhoutte (1988).

MDL 72222, a selective and potent 5-HT_3 antagonist (Fozard, 1984), did not antagonize responses to 5-HT, providing strong evidence that 5-HT_3 receptors were not present in this tissue. Furthermore, the contractions to 2-methyl-5-HT, a selective 5-HT_3 receptor agonist (Bradley et al. 1986), were not blocked by MDL 72222 (10^{-6}M) but were effectively blocked by ketanserin (10^{-8}M). The dissociation constant of ketanserin vs. 2-methyl-5-HT (2.88nM) was similar to that of ketanserin vs. 5-HT (2nM), suggesting that the 2-methyl-5-HT-induced contraction in the uterine artery was mediated by 5-HT_2 receptors.

In summary, 5-HT and DOM are potent agonists in producing contraction via 5-HT_2 receptors in the late pregnant ovine uterine artery. Activation of alpha adrenergic receptors are not involved in the response to 5-HT. The presence of 5-HT_{1A} receptors in this tissue was unlikely since ketanserin antagonized contractions to 8-OH-DPAT. In contrast to their agonist properties at central 5-HT receptors, the phenylpiperazine derivatives, TFMPP, mCPP and 2-MPP acted as antagonists at 5-HT_2 receptors in the uterine artery. 5-HT_3 receptors are not present in this tissue.

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SECTION III. 5-HT_2 RECEPTOR-STIMULATED CALCIUM INFLUX IN THE OVINE UTERINE ARTERY IN LATE PREGNANCY[1]

Abstract

The effects of 5-hydroxytryptamine (5-HT) and 2,5-dimethoxy-4-methyl-amphetamine (DOM) on the Ca^{2+} influx in the ovine uterine artery in late pregnancy have been studied by measuring the $^{45}Ca^{2+}$ uptake. Both 5-HT and DOM induced concentration-dependent (2.5×10^{-8} - 2.5×10^{-5}M) rises in $^{45}Ca^{2+}$ uptake in the uterine artery, from a basal level of 30.5 ± 3.5 μMoles/kg wet tissue to peak levels of 91.1 ± 9.2 and 84.2 ± 8.1 μMoles/kg wet tissue, respectively. Ketanserin inhibited the Ca^{2+} influx evoked by 5-HT and DOM. The dissociation constants (k_B) of ketanserin, tested against 5-HT and DOM, were not significantly different, indicating that the Ca^{2+} influx produced by both agonists are mediated by the same 5-HT receptor, i.e., 5-HT_2 receptor. Methiothepin (2.5×10^{-7}M) also inhibited 5-HT (2.5×10^{-6}M) induced Ca^{2+} influx by 86%. No antagonism has been found with 2.5×10^{-6}M of 3-tropanyl-3,5-dichlorobenzoate (MDL 72222). The contraction elicited by 5-HT (10^{-6}M) and DOM (10^{-6}M) was blocked by D600 (10^{-5}M) which also blocked the contraction produced by KCl (90mM). Amrinone (10^{-5}M) showed no inhibitory effect of these contractions. In accord with the results of the contraction study, D600 (2.5×10^{-6}M) blocked the Ca^{2+} influx stimulated by 5-HT (2.5×10^{-6}M) and amrinone (2.5×10^{-5}M) failed

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[1]Submitted to Eur. J. Pharmacol. as a research paper by Lubo Zhang and Donald C. Dyer.

to inhibit 5-HT-induced Ca^{2+} influx. These data clearly showed that 5-HT induced Ca^{2+} in this preparation was mediated by voltage-dependent Ca^{2+} channels. The further evidence came from the inhibition of nifedipine (2.5×10^{-5}M) on the 5-HT-induced Ca^{2+} influx. Sodium nitroprusside was also shown to inhibit the 5-HT-induced Ca^{2+} influx, which indicated that at least part of its inhibitory effect on the vasoconstriction evoked by 5-HT was due to the inhibition of the 5-HT-induced Ca^{2+} influx.

Introduction

The calcium ions that initiate a mechanical response in smooth muscle cells may be mobilized from calcium reservoirs within the cell and/or from the calcium pool (both free and loosely bound calcium) in the external environment (VanBreeman et al., 1982). The relative dependence of the contraction on these two calcium sources has been shown to vary with agonist, concentration of agonist, blood vessels and species studied (Fleckenstein, 1977; Kalsner et al., 1970; Weiss, 1977). A series of calcium channel blockers have helped to unravel the possible role of the extracellular calcium in the agonist-induced vasoconstrictions. It has been proposed that 5-hydroxytryptamine (5-HT) could stimulate depolarization of the smooth muscle membranes (Fujiwara et al., 1982) and induce contractions mediated by influx of the extracellular calcium (Berta et al., 1986; Capponi; et al., 1987; Nakaki et al., 1985; Ratz and Flaim, 1984).

It has been well demonstrated that 5-HT produced a vasoconstriction

in many blood vessels (Van Nueten et al., 1981, 1984; Leysen et al., 1984; Maayani et al., 1984; Zhang and Dyer, 1989a, 1989b). Major advances in 5-HT receptor classification have undoubtedly arisen as a result of new drug tools recently identified such as the potent pharmacologically selective antagonists ketanserin and MDL 72222 (3-tropanyl-3,5-dichlorobenzoate). In our previous study, we demonstrated that 5-HT and 2,5-dimethoxy-4-methyl-amphetamine (DOM) were potent agonists mediating vasoconstriction in ovine uterine artery by acting on 5-HT_2 receptors (Zhang and Dyer, 1989a). We have now demonstrated that 5-HT and DOM stimulated voltage-dependent calcium channel mediated Ca^{2+} influx in ovine uterine artery by measuring the smooth muscle $^{45}Ca^{2+}$ uptake. Additionally, several serotonergic antagonists have been used to determine the subtype of 5-HT receptors linked to 5-HT-induced Ca^{2+} influx.

Methods

Uterine arteries from pregnant mixed breed sheep were helically cut and prepared as previously described (Zhang and Dyer, 1989a). The arterial strips were suspended in 10-ml isolated organ baths, maintained at 37°C and bathed with a modified Krebs-Henseleit (Krebs) solution of the following composition (mM): NaCl, 115.21; KCl, 4.70; $CaCl_2$, 1.80; $MgSO_4$, 1.16; KH_2PO_4 1.18; $NaHCO_3$, 22.14; dextrose, 7.88. Disodium ethylenediamine tetracetic acid (EDTA 0.03 mM) was added to suppress oxidation of amines. The Krebs' solution was oxygenated with a mixture of oxygen-carbon dioxide (95:5). The tissues were equilibrated under 1g

of tension for 90 min before initiating the experiment, and contractions were recorded isotonically.

Effects of Ca^{2+} channel blockers on KCl and agonist-induced contractions

After the 90 min equilibration period a concentration-response relationship was developed by adding a single concentration of an agonist to a series of isolated muscle baths containing arterial strips prepared from the same uterine artery. Initially all tissues were exposed to KCl 90mM, a concentration which produced approximately 80% of the maximum response, and the responses to all agonists were compared to the KCl (90mM) response. This was followed by adding a single concentration of the agonist under study to establish a control response. The tissues were then repeatedly washed and the tissues allowed to relax to the original baseline. Tissues were then exposed to D600 (10^{-6}M) or amrinone (10^{-5}M) for 30 min. A second exposure to the same concentration of the agonist was obtained and the contraction produced compared to the control. In another set of experiments the effect of D600 on contractions developed to agonists was determined by adding the D-600 at the peak of the contraction.

Ca^{2+} uptake studies

Calcium influx was measured by using a technique previously reported by Stice et al. (1987). In brief, the segment of the uterine artery of the same positional location from different animals was utilized for Ca^{2+} influx determinations. The uterine artery was helically cut into strips 10mm long and 2 mm wide. A 1 g weight was used to apply a constant tension to the arterial smooth muscle. After equilibration, each strip was exposed to a different concentration of

agonist, i.e., 5-HT (10 min) or DOM (15 min), in a $^{45}Ca^{2+}$ buffer (0.5 μCi/ml). Where appropriate, inhibitors were added 30 min prior to the agonist challenge. Thereafter, the strips were emersed in cold (4°C) lanthanum chloride (10^{-3}M) solution for 60 min. The tissues were then blotted dry on filter paper, weighed and placed in a separate scintillation vial containing 0.5 ml of protosol (NEN Research Products, Boston, MA) and heated at 60°C for 2 hrs to dissolve the tissues. Ten milliliters of Scinti Verse (Fisher Scientific, Fair Lawn, New Jersey) was added to each vial. Radioactivity in the tissue was detected by scintillation counting. Ca^{2+} uptake was determined by the apparent tissue content of $^{45}Ca^{2+}$ with adjustment for its original specific activity and normalized for the weight of each tissue. Nonspecific binding of ^{45}Ca to the tissue was determined for each uterine artery by incubating the uterine artery strip with 4 ml of aerated Ca^{2+}-free buffer containing 10^{-3}M $LaCl_3$ for 30 min before exposure to 0.5 μCi/ml of ^{45}Ca. Nonspecific binding, which averaged 20.3 ± 4.7% of specific uptake, was subtracted from total ^{45}Ca uptake for each artery to obtain specific uptake. The influx is referred to as the difference between the uptake measured in the presence of stimulant and the corresponding control. Inhibitory activity of a drug is expressed as a percent of maximal influx induced by the stimulant in question.

Data analysis

Estimates of EC_{50} for 5-HT and DOM in Ca^{2+} uptake studies were obtained by fitting Equation 1: $R = RmaxD/(D + EC_{50})$ to the observed response R as a function of drug concentration [D] using a computer program which is based on procedures outlined by Tallarida and Murray

(1981). To obtain estimates of IC_{50}s for the experiments in which the 5-HT and DOM responses were inhibited by ketanserin, Equation 1 was fitted so that the Rmax was a constant equal to 100% of the uninhibited responses to 5-HT or DOM, the EC_{50} being equivalent to IC_{50}.

For inhibition experiments, ketanserin IC_{50}s were converted to dissociation constant (k_B) values using the Cheng-Prussof Equation 2 (Cory et al., 1986): $k_B = IC_{50}/(1 + [Agonist]/EC_{50})$ in which [Agonist] is the concentration (2.5×10^{-6}M) of 5-HT and/or DOM used in the inhibition experiments and EC_{50} is the mean value of those determined for 5-HT and/or DOM in this study. This equation is based on a model of competitive antagonism which is obtained by using ketanserin in the present study.

Data were expressed as means ± SE. For each experiment, n refers to the number of sheep from which the uterine artery was taken. The Student's t-test was used for statistical analysis of the difference of means.

Drugs and chemicals

The following drugs and chemicals were used; serotonin creatinine sulfate, sodium nitroprusside (Sigma Chemical Co., St. Louis, MO); R(-)-2,5-dimethoxy-4-methyl-amphetamine (DOM) (National Institute of Drug Abuse, Rockville, MD); l-epinephrine bitartrate, l-norepinephrine bitartrate (Calbiochem Behring Corp., La Jolla, CA); methiothepin maleate (Hoffmann-LaRoche, Nutley, NJ); ketanserin tartrate (Janssen, Beerse, Belgium); 3-tropanyl-3,5-dichlorobenzoate (MDL 72222) (Res. Biochem. Inc., Natick, Massachusetts); Nifedipine (Pfizer, Brooklyn, NY); D600 (A. G. Knoll Pharmaceutical Laboratories, Ludwigshafen, W.

Germany); amrinone (Sterling Winthrop, Rensselaer, NY); and $^{45}Ca^{2+}$ (31.18 mCi/mg; New England Nuclear, Boston, MA).

Results

Effects of D600 and amrinone on KCl and the agonist-induced contractions

Single concentration (10^{-6}M) of 5-HT and DOM elicited contractions that were 112 and 114 percent, respectively, of that produced by KCl 90 mM. Following exposure of the tissues to D600 (10^{-5}M) for 15 min, responses to KCl, 5-HT and DOM were blocked (Fig. 1). No antagonism by amrinone (10^{-5}M) was observed (Fig. 1).

D600 (10^{-6}M) totally blocked the 5-HT and DOM-induced contractions when added after the contractions have reached their plateaus, but incompletely blocked those to KCl, norepinephrine and epinephrine when added at the same manner (Fig. 2).

Effects of 5-HT and DOM on Ca^{2+} uptake

5-HT (2.5×10^{-8} to 2.5×10^{-5}M) induced concentration-dependent increases of Ca^{2+} uptake in uterine artery from resting values of 30.5 ± 3.5 μMoles/kg wet tissue to a peak of 91.1 ± 9.7 μMoles/kg wet tissue. The concentration-response curve of 5-HT was showed in Fig. 3. EC_{50} of 5-HT in stimulating Ca^{2+} uptake was determined to be $4.57 \pm 1.33 \times 10^{-7}$M. Similar results were obtained to DOM (Fig. 3). DOM (2.5×10^{-8}M to 2.5×10^{-5}M) increased Ca^{2+} uptake dose-dependently from the resting level of 30.5 ± 3.5 μMoles/kg wet tissue to the peak level of 84.4 ± 7.9 μMoles/kg wet tissue, which was not significantly different from that of 5-HT. The EC_{50} of DOM was determined to be $2.75 \pm 1.15 \times 10^{-7}$M and was

Fig. 1. Effects of D600 and amrinone on the vasoconstriction responses to KCl, 5-HT and DOM in ovine pregnant uterine artery. Both the D600 and amrinone were equilibriated with the tissue for 30 min prior to challenge to the agonists. The results are presented as $\bar{x} \pm$ S.E. (N = 3). The treatment groups are compared to their control groups. ** $P < 0.01$

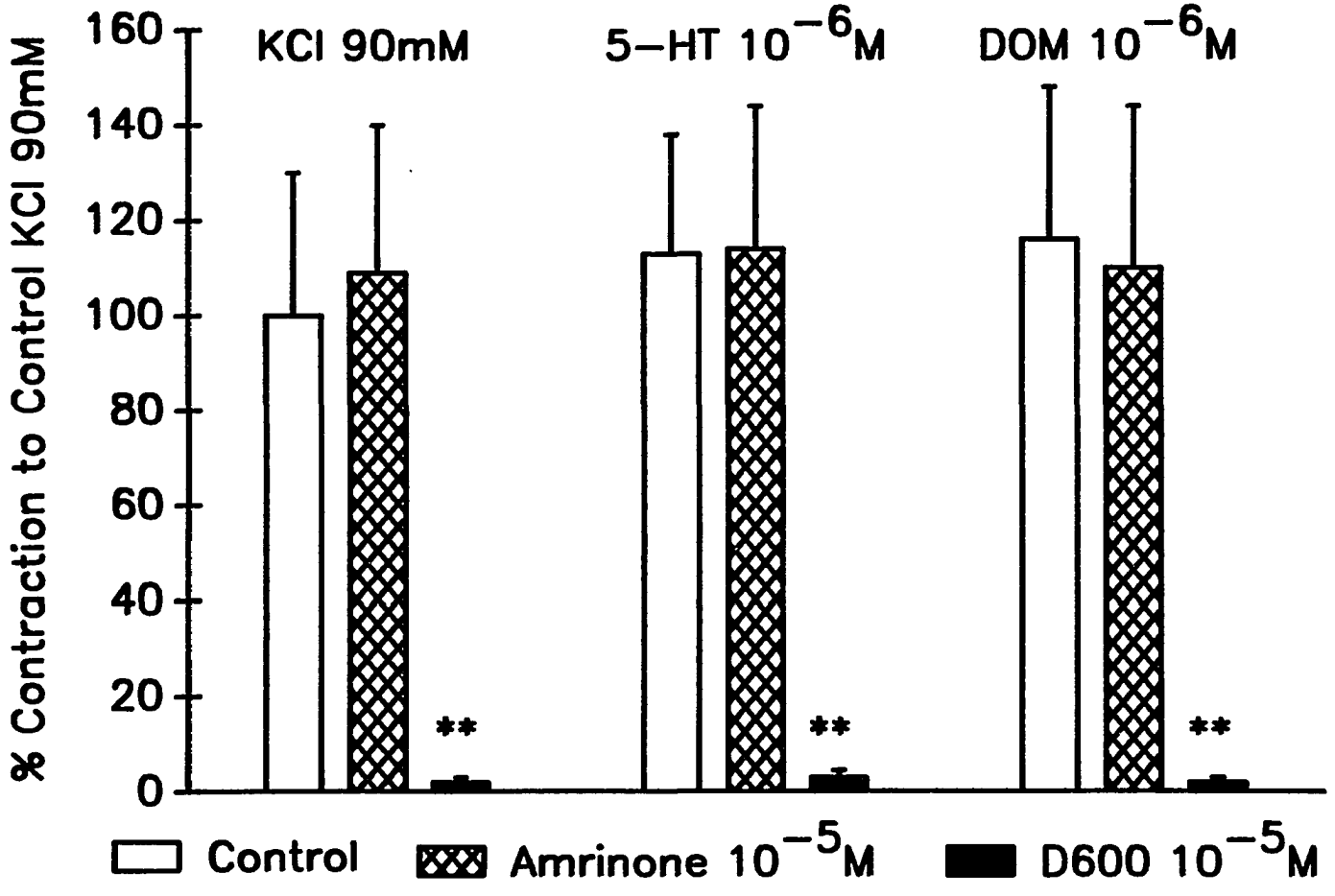

% Contraction to Control KCl 90mM
160
140
120
100
80
60
40
20
0
KCl 90mM
5–HT 10^{-6}M
DOM 10^{-6}M
**
**
**
Control
Amrinone 10^{-5}M
D600 10^{-5}M

Fig. 2. Effects of D600, when added at the top of the responses, on the vasoconstriction responses to KCl, 5-HT, DOM, NE or EPI in ovine pregnant uterine artery. The results are presented as $\bar{x} \pm$ S.E. (N = 3). The treatment groups are compared to their control groups. * $P < 0.05$, ** $P < 0.01$

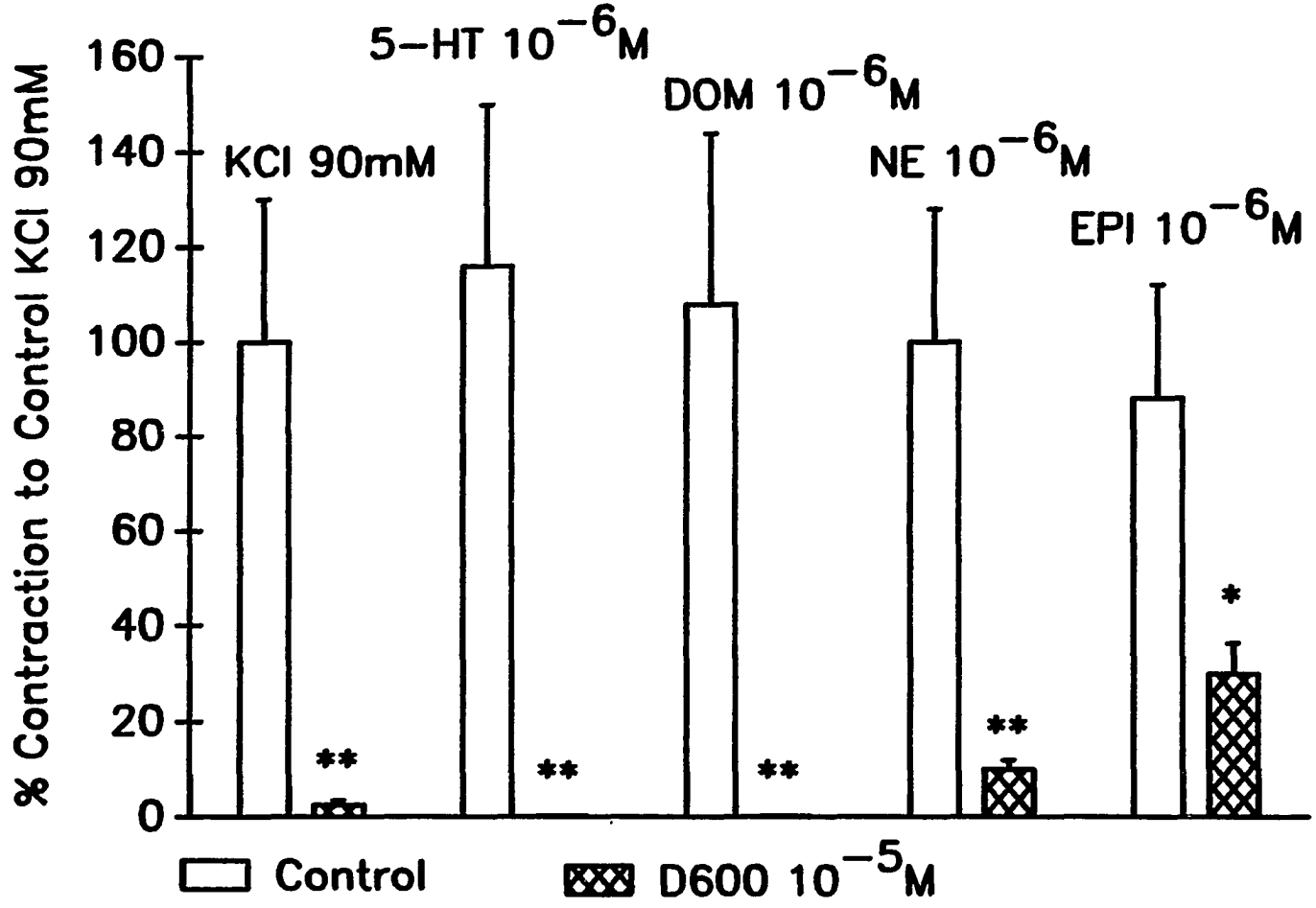
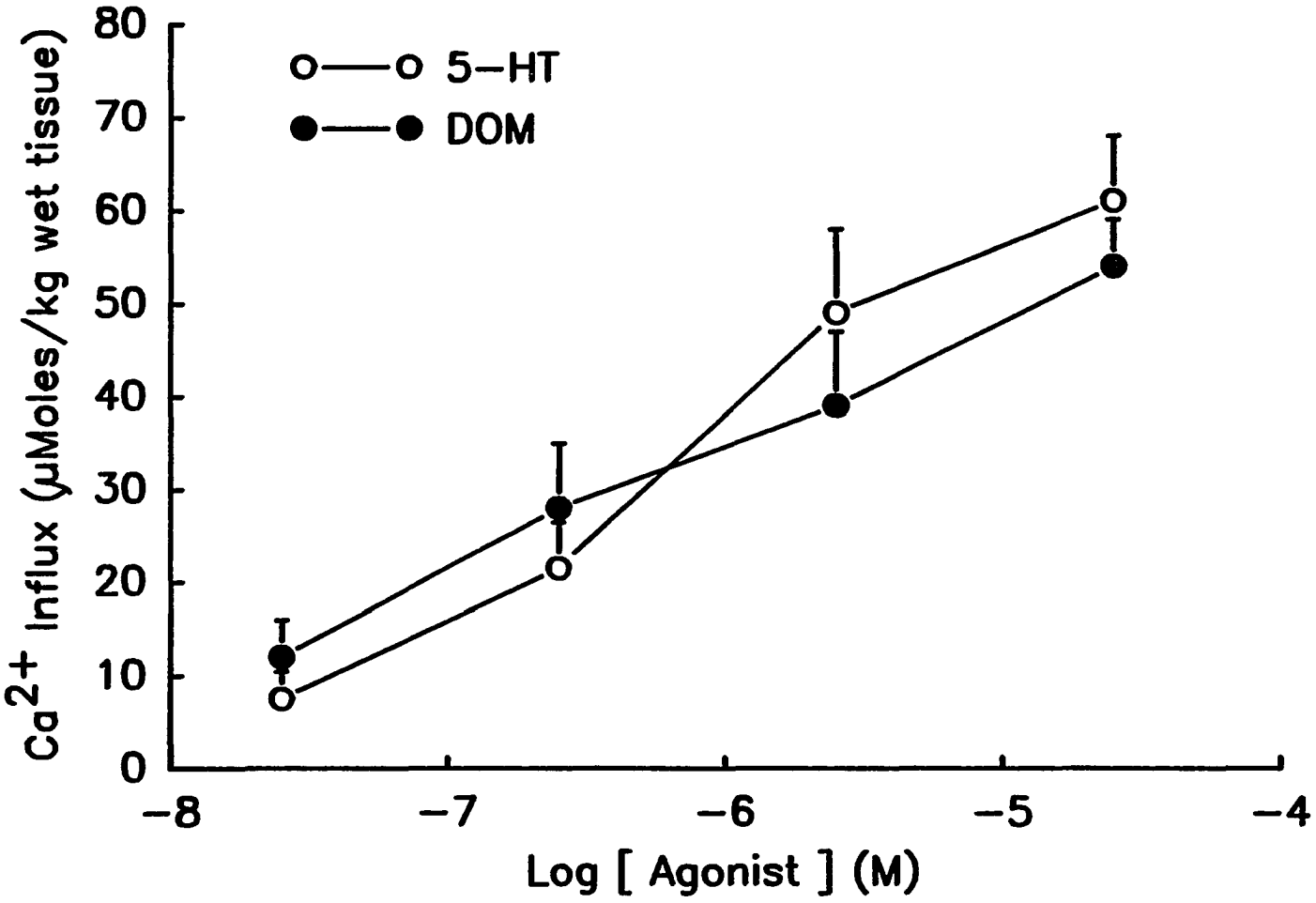

% Contraction to Control KCl 90mM
160
140
120
100
80
60
40
20
0
KCl 90mM
5-HT 10^{-6}M
DOM 10^{-6}M
NE 10^{-6}M
EPI 10^{-6}M
**
**
**
**
*
Control
D600 10^{-5}M

Fig. 3. Effects of 5-HT and DOM on the Ca^{2+} influx in ovine pregnant uterine artery. The results are presented as $\bar{x} \pm$ S.E. (N = 6)


80
70
60
50
40
30
20
10
0
Ca^{2+} Influx (μMoles/kg wet tissue)
o—o 5-HT
●—● DOM
−8
−7
−6
−5
−4
Log [Agonist] (M)

not significantly different from that of 5-HT.

Effects of serotonergic antagonists on 5-HT and DOM induced Ca^{2+} influx

The ability of three serotonergic antagonists to inhibit the 5-HT elicited increase in Ca^{2+} uptake was investigated. Two of these compounds, the 5-HT_2 selective antagonist ketanserin and 5-HT_1 and 5-HT_2 antagonist methiothepin, were potent inhibitors of Ca^{2+} influx induced by 2.5 x 10^{-6}M 5-HT (Fig. 4). Exposure of the tissues to ketanserin (2.5 x 10^{-7}M) and methiothepin (2.5 x 10^{-7}M) for 30 min, significantly ($P < 0.01$) decreased Ca^{2+} uptake by 5-HT (2.5 x 10^{-6}M) from 79 ± 9.1 μMoles/kg wet tissue to 39 ± 5.1 μMoles/kg wet tissue and 39 ± 4.2 μMoles/kg wet tissue, respectively. Ketanserin produced a concentration-dependent inhibition on Ca^{2+} influx elicited by 5-HT and/or DOM (Fig. 5). The two ketanserin inhibition curves were essentially superimposed. The IC_{50}s of ketanserin vs. 5-HT and DOM were determined to be 2.60 ± 0.98 x 10^{-8}M and 3.00 ± 1.28 x 10^{-8}M, respectively. Ketanserin is a competitive 5-HT_2 antagonist. In the previous study, we showed that ketanserin competitively antagonized the contractions produced by 5-HT and DOM in the ovine uterine artery (Zhang and Dyer, 1989a). The K_B values for ketanserin in inhibiting Ca^{2+} influx induced by 5-HT and DOM were calculated using Equation 2 and were found to be 4.02 ± 1.42 x 10^{-9}M and 2.97 ± 1.25 x 10^{-9}M, respectively. The selective 5-HT_3 antagonist MDL 72222 (2.5 x 10^{-6}M) produced no antagonism on the Ca^{2+} influx induced by 5-HT (2.5 x 10^{-6}M) (Fig. 4).

Effects of Ca^{2+} channel blockers on 5-HT induced Ca^{2+} influx

The resting Ca^{2+} uptake measured with $^{45}Ca^{2+}$ was 30.5 ± 3.5 μMoles Ca^{2+}/kg wet tissue per 10 min. 5-HT (2.5 x 10^{-8}M) increased the Ca^{2+}

Fig. 4. Effects of serotonergic antagonists on the 5-HT-induced Ca^{2+} uptake in ovine pregnant uterine artery. Met., methiothepin; ket., ketanserin; MDL., MDL 72222 were equilibriated with the tissue for 30 min prior to adding 5-HT. The results are presented as $\bar{x} \pm$ S.E. (N = 6). The serotonergic antagonist treatment groups are compared to the 5-HT alone treatment group. ** $P < 0.01$

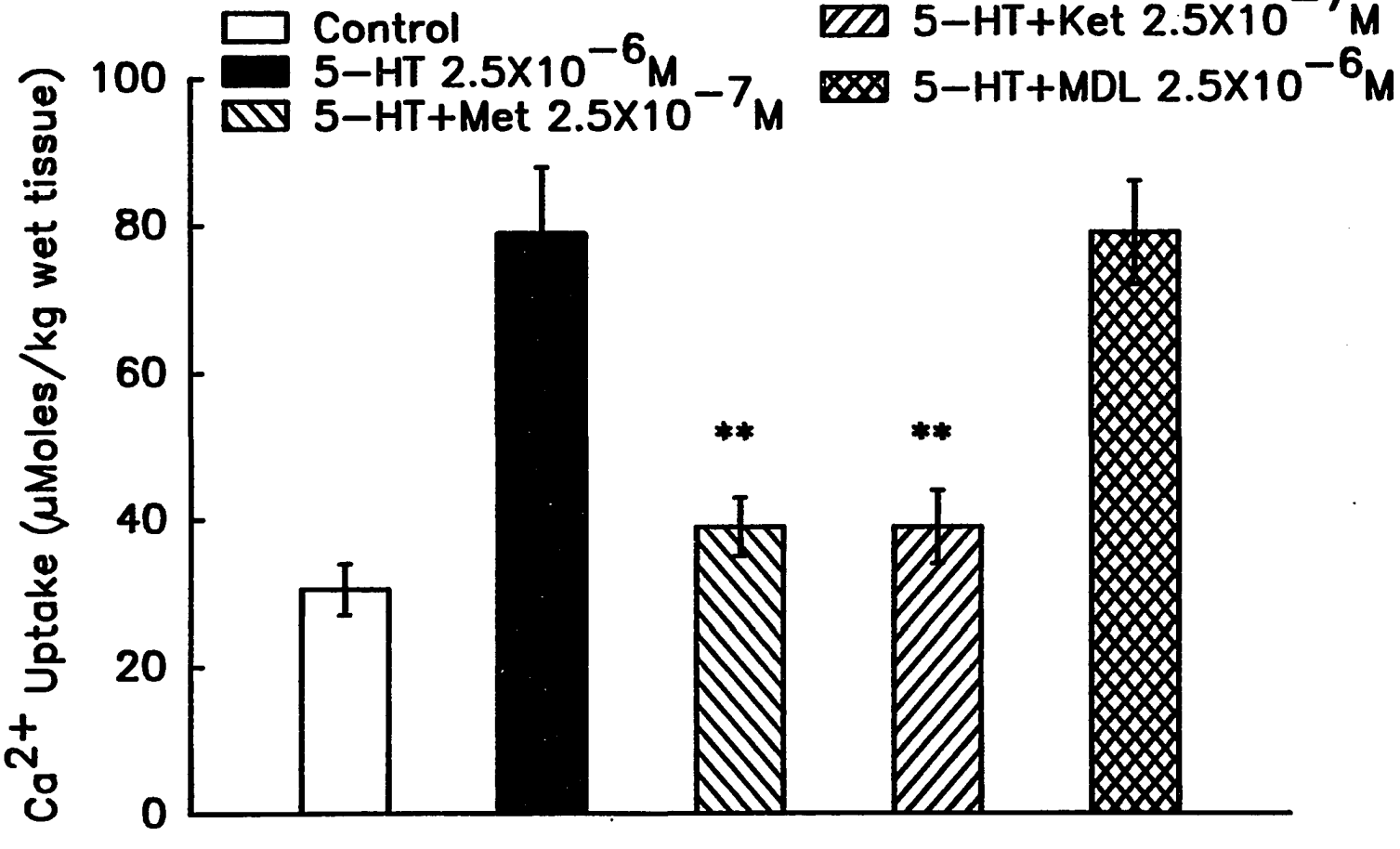
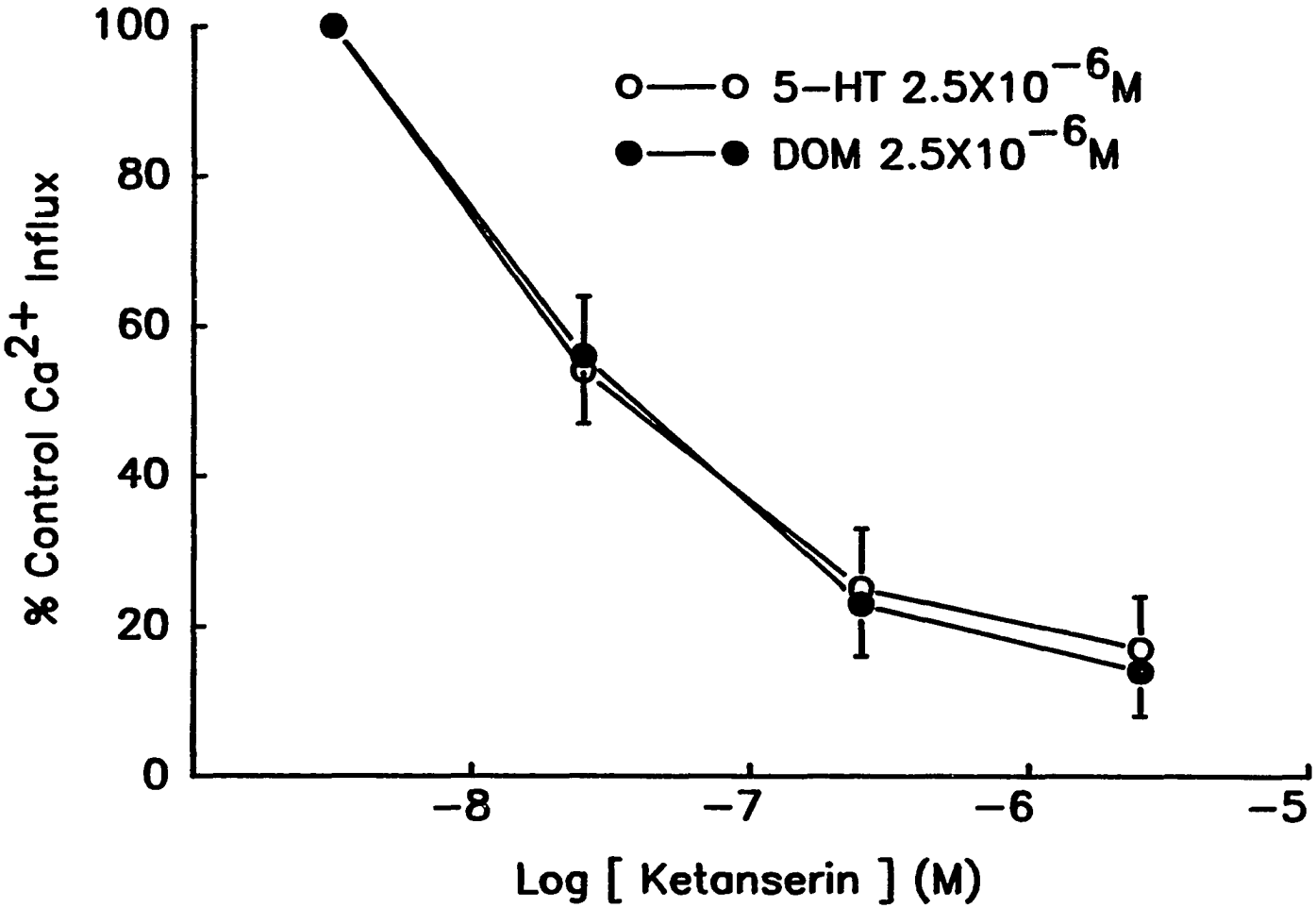

Control
5–HT 2.5X10^{-6}M
5–HT+Met 2.5X10^{-7}M
5–HT+Ket 2.5X10^{-7}M
5–HT+MDL 2.5X10^{-6}M
Ca^{2+} Uptake (μMoles/kg wet tissue)
100
80
60
40
20
0
**
**

Fig. 5. Effects of ketanserin on the Ca^{2+} influx induced by 5-HT and DOM in ovine pregnant uterine artery. The ketanserin was equilibriated with the tissue for 30 min prior to challenge with the agonists. The results are presented as $\bar{x} \pm$ S.E. (N = 6)

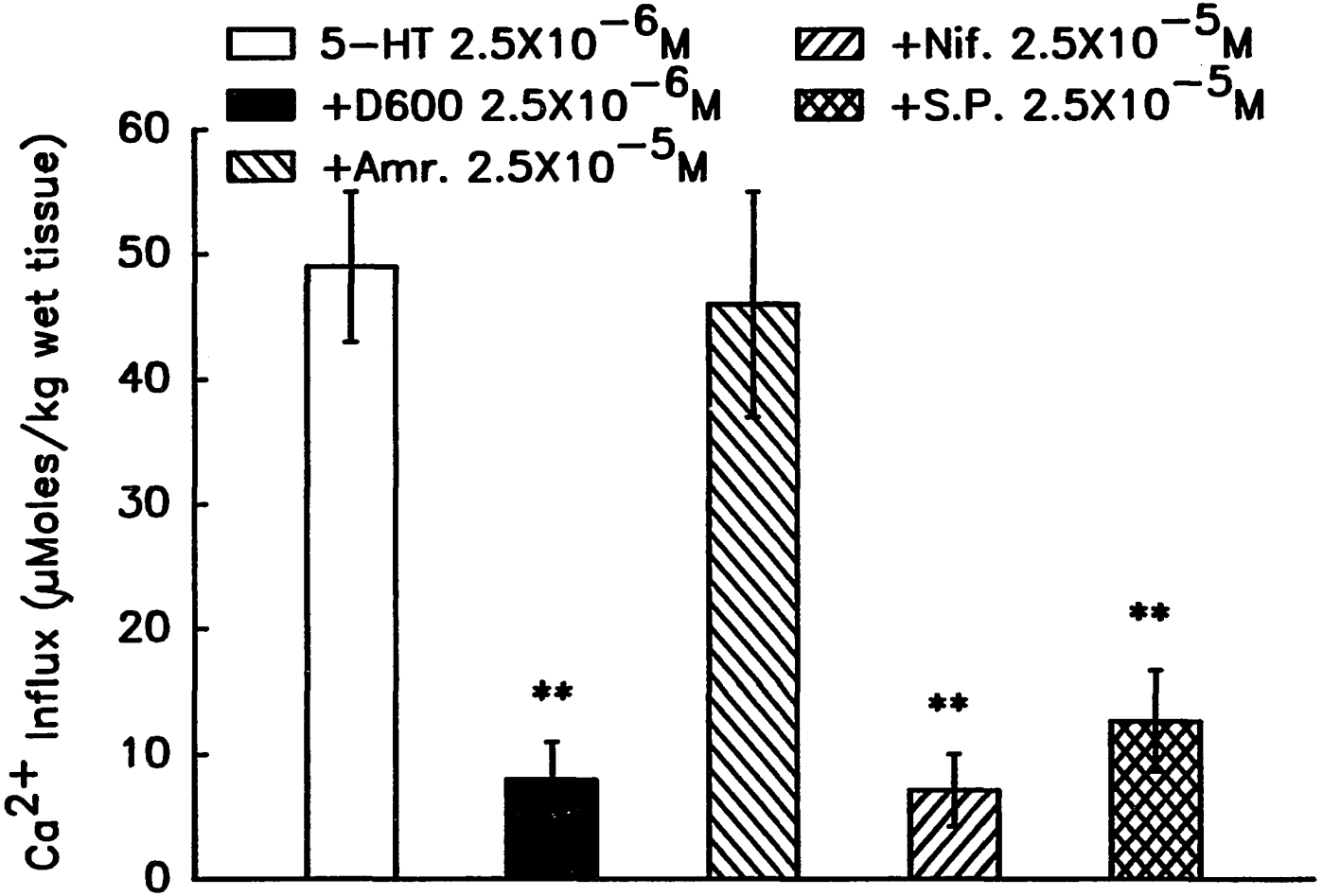

100
80
60
40
20
0
% Control Ca^{2+} Influx
o—o 5-HT $2.5X10^{-6}M$
●—● DOM $2.5X10^{-6}M$
-8
-7
-6
-5
Log [Ketanserin] (M)

uptake to 79.6 ± 9.2 μMoles Ca^{2+}/kg wet tissue per 10 min. The Ca^{2+} influx induced by 5-HT was referred to as the difference between the uptake measured in the presence of 5-HT and the corresponding resting level. In this case 5-HT (2.5×10^{-6}M) stimulated Ca^{2+} influx was 49.1 ± 6.1 μMoles Ca^{2+}/kg wet tissue per 10 min. Fig. 6 showed the effects of Ca^{2+} channel blockers on the Ca^{2+} influx induced by 5-HT (2.5×10^{-6}M). D600 (2.5×10^{-6}M) decreased the 5-HT-stimulated Ca^{2+} influx to 7.4 ± 2.1 μMoles Ca^{2+}/kg ($P<0.01$). The same concentration of nifedipine showed no inhibitory effect on the 5-HT-induced Ca^{2+} influx. However, increasing the concentration of nifedipine (2.5×10^{-5}M) 10 times produced a similar inhibition as that to D600 (Fig. 6). Sodium nitroprusside (2.5×10^{-5}M) also decreased the 5-HT-stimulated Ca^{2+} influx with its inhibitory effect being slightly less than that produced by the same concentration of nifedipine (Fig. 6). No antagonism of the 5-HT-induced Ca^{2+} influx was observed with 2.5×10^{-5}M amrinone (Fig.6).

Discussion

Extracellular Ca^{2+} has been proposed to play a role in the vascular contractions produced by 5-HT receptor activation (Berta et al., 1986; Capponi et al., 1987; Nakaki et al., 1985; Ratz and Flaim, 1984). Experiments in the present study clearly demonstrate that extracellular Ca^{2+} was involved in 5-HT mediated responses in the ovine uterine artery. 5-HT produced a concentration-dependent increase in Ca^{2+} uptake in the uterine artery. The EC_{50} (0.46μM) of 5-HT in mediating Ca^{2+} influx in this preparation was comparable to its EC_{50} (0.13 μM) for

Fig. 6. Effect of Ca^{2+} channel blockers on the 5-HT-induced Ca^{2+} influx in ovine pregnant uterine artery. Amr., amrinone; Nif., nifedipine; S.P., sodium nitroprusside were equilibriated with the tissue for 30 min prior to challenge with the 5-HT. The results are presented as $\bar{x} \pm$ S.E. (Control, D600 and amrinone treatment groups, N = 6, nifedipine and sodium nitroprusside treatment groups, N = 3). The Ca^{2+} channel blocker treatment groups are compared to 5-HT control group. ** $P < 0.01$


5−HT 2.5X10^{-6}M
+D600 2.5X10^{-6}M
+Amr. 2.5X10^{-5}M
+Nif. 2.5X10^{-5}M
+S.P. 2.5X10^{-5}M
Ca^{2+} Influx (μMoles/kg wet tissue)
60
50
40
30
20
10
0
**
**
**

producing contractions (Zhang and Dyer, submitted for publication). These results suggest that the 5-HT-induced Ca^{2+} influx and contractions are causally related.

5-HT has been found to stimulate phosphatidylinositol metabolism in the cerebral cortex (Berridge et al., 1982; Brown et al., 1984; Conn and Sanders-Bush, 1984, 1987; Pierce and Peroutka, 1988) and in vascular smooth muscle (Doyle et al., 1986; Roth et al., 1984). The phosphatidylinositol turnover stimulated by 5-HT was via 5-HT_2-receptors. One can therefore assume that in vascular smooth muscle cells as in the cerebral cortex serotonin acts through activation of a membranous phospholipase C. The breakdown of phosphoinositides generates inositol 1,4,5-triphosphate (IP_3), which is known to mobilize calcium from intra-cellular stores and 1,2-diacylglycerol, which activates the Ca^{2+}-sensitive, phospholipid-dependent protein kinase C (Rasmussen et al., 1987). It has been shown that 5-HT stimulated phosphatidylinositol turnover in rat aorta and that in this tissue the mechanism of the 5-HT-induced contraction required the participation of the opening of a voltage-gated calcium channel and the stimulation of a phosphatidylinositol-specific phospholipase C (Nakaki et al., 1985; Roth et al., 1986). Recent evidence indicates that a rise in cytosolic Ca^{2+} can also activate phospholipase C (Eberhard and Holz, 1987; Fisher and Agranoff, 1981; Kendall and Nahorski, 1984, 1985). Ca^{2+}-activated phospholipase C may represent a positive feedback system for Ca^{2+}; that is, small increases in cytosolic Ca^{2+} induced by Ca^{2+} influx across the plasma membrane may result in higher cytosolic Ca^{2+} concentrates due to IP_3-induced release of Ca^{2+} from intracellular stores. The IP_3 produced

in this way could also act on the plasma membrane to increase Ca^{2+} influx as it does on the membrane of the responsive fraction of endoplasmic reticulum (Kuno and Gardner, 1987; Slack et al., 1986). The activation of phospholipase C by Ca^{2+} may also provide a mechanism for diacylglycerol generation and protein kinase C activation following Ca^{2+} influx. Thus, a rise in cytosolic Ca^{2+} initially induced by 5-HT in this study may contribute to the activation of phospholipase C which in turn will stimulate phosphatidylinositol turnover as demonstrated by others.

There are two major routes by which calcium ions from the extracellular pool may traverse the cell membrane and thereby gain access to the contractile apparatus. One pathway is the receptor-operated calcium channel system; the other is the voltage-dependent calcium channel system (Bolton, 1979; Meisheri et al., 1981; VanBreemen et al., 1982). D600, an inhibitor of the voltage-dependent Ca^{2+} channel (Bolton, 1979; Meisheri et al., 1981), potently inhibited Ca^{2+} influx mediated by 5-HT. Amrinone has been demonstrated as an inhibitor on norepinephrine- or phenylephrine-induced Ca^{2+} influx in the rabbit aorta and the pig uterine artery (Meisheri et al., 1981; Stice et al., 1987). The k^{+}-induced Ca^{2+} influx was not affected by amrinone. These authors suggested that amrinone may act on the receptor-operated calcium channel. In the present study, amrinone ($2.5 \times 10^{-5}M$) showed no inhibition on 5-HT-induced Ca^{2+} influx. These results indicate that 5-HT-induced Ca^{2+} influx in this tissue is mediated by the voltage-dependent calcium channels. Further evidence comes from the contraction studies in the uterine artery in which D600 ($10^{-5}M$) blocked contractions

evoked by KCl, 5-HT and DOM but amrinone (10^{-5}M) had no effect. It has been demonstrated that the vascular contraction elicited by 5-HT involves depolarization of the smooth muscle membranes (Fujiwara et al., 1982) which could open the voltage-dependent calcium channel (Brown et al., 1981; Hille, 1984).

One of the interesting findings was that when added after the agonist-induced contractions had reached plateaus, D600 (10^{-5}M) totally blocked the contractions elicited by 5-HT and DOM but only partially blocked those by NE and EPI. It has been demonstrated that NE-induced Ca^{2+} influx could include both receptor-operated and voltage-dependent Ca^{2+} channel mechanisms (Hester, 1988; Nelson et al., 1988). On the other hand, D600 has been demonstrated as an apparent competitive antagonist of 5-HT receptors in rabbit isolated aortas (Auguet et al., 1986). It has been proposed that D600 could be termed a 'Ca^{2+}/5-HT-antagonist' (DeFeudis, 1984).

Nifedipine is also an inhibitor of the voltage-dependent Ca^{2+} channel (Kanmura et al., 1983). In the present study, nifedipine (2.5 x 10^{-5}M) inhibited 86% of 5-HT-induced Ca^{2+} influx. The concentration of nifedipine used seems very critical since 2.5 x 10^{-6}M nifedipine showed no inhibition on 5-HT-mediated Ca^{2+} influx. This is in accord with the finding by Capponi et al. (1987) in which they found that 10^{-6}M nifedipine was unable to prevent the intracellular free Ca^{2+} rise triggered by 5-HT challenge. The finding that nifedipine at higher concentrations can inhibit 5-HT-induced Ca^{2+} influx further confirms that voltage-dependent Ca^{2+} channel mechanisms are involved in Ca^{2+} influx evoked by 5-HT in the ovine uterine artery from pregnancy.

The mechanisms of action of sodium nitroprusside may include membrane hyperpolarization, inhibition of Ca^{2+} uptake, increase in Ca^{2+} extrusion or sequestration, inhibition of contractile elements, or inhibition of receptor-linked phosphoinositide breakdown (Karaki et al., 1984, 1988; Popescu et al., 1985; Rapoport, 1986). Sodium nitroprusside potently inhibited the contractile response to NE in human pregnant uterine arteries (Nelson and Suresh, 1988) and the contractions to 5-HT in ovine pregnant uterine arteries (Isla and Dyer, submitted for publication). In the present study sodium nitroprusside inhibited the 5-HT-induced Ca^{2+} influx. This finding indicates that the inhibitory effect of sodium nitroprusside on 5-HT-evoked contraction in the pregnant uterine artery may be at least partly due to inhibition of the 5-HT-induced Ca^{2+} influx in this preparation.

Ketanserin, a selective 5-HT_2 receptor antagonist (Leysen et al., 1981; Van Nueten et al., 1981; Leysen et al., 1982) potently inhibited the Ca^{2+} influx evoked by 5-HT and DOM. This result is in accord with the finding by Capponi et al. (1987) in which ketanserin (10^{-6}M) has been shown to completely block the free Ca^{2+} rise induced by 5-HT. Our recent studies have demonstrated that ketanserin competitively antagonized the contraction produced by 5-HT and DOM in the ovine uterine artery from pregnancy (Zhang and Dyer, 1989a). The dissociation constants for ketanserin vs. 5-HT (4.02 nM) and DOM (2.97 nM) determined in this study were in accord with those of ketanserin against 5-HT (2 nM) and DOM (4 nM) in our recent contraction studies in the ovine uterine artery (Zhang and Dyer, 1989a) and were comparable to its affinity (k_i = 2.1 nM) for 5-HT_2 binding sites (Leysen et al., 1981;

Leysen et al., 1982) and its dissociation constants (7 nM to 0.2 nM) reported in other vessels by other authors (Cohen et al., 1983; Cohen, 1986; Humphrey, 1984; Van Nueten et al., 1982; Van Nueten et al., 1981). This indicates that both 5-HT and DOM induced Ca^{2+} influx in the uterine artery and contractions to these agonists are related to activation of the same receptor, i.e., the 5-HT_2 receptor.

Methiothepin is a nonselective antagonist with affinity for both 5-HT_1 and 5-HT_2 binding sites (Martin and Sanders-Bush, 1982; Engel et al., 1983). It produced a potent inhibition on 5-HT-induced Ca^{2+} influx in this study. It was also a strong antagonist of 5-HT-induced contraction in the ovine uterine artery (Zhang and Dyer, submitted for publication). The presence of '5-HT_1-like' subtype receptors in this tissue is unlikely since we have demonstrated that 5-HT-induced contractions in the ovine uterine artery was mediated by 5-HT_2 receptors (Zhang and Dyer, 1989a). The 5-HT_3 receptor antagonist MDL 72222 (Fozard, 1984) did not antagonize 5-HT-induced Ca^{2+} influx, indicating that 5-HT_3 receptor is not present in this tissue. This supports our previous finding in which MDL 72222 was found not to antagonize contractions to 5-HT (Zhang and Dyer, 1989a).

In summary, 5-HT and DOM stimulated concentration-dependent Ca^{2+} influx in ovine pregnant uterine artery, implying that the extracellular Ca^{2+} played an important role in the responses mediated by 5-HT receptors. It was demonstrated that 5-HT_2 receptors were involved in the 5-HT-induced Ca^{2+} influx. The presence of 5-HT_1 receptors in this tissue is unlikely. 5-HT_3 receptors were not involved in the 5-HT-induced Ca^{2+} influx. D600 and nifedipine blocked the 5-HT-induced Ca^{2+}

influx in the ovine uterine artery from pregnancy and amrinone had no effect. These results suggest that 5-HT-induced Ca^{2+} influx in this tissue was mediated by voltage-dependent Ca^{2+} channel. The inhibitory effect by sodium nitroprusside of the 5-HT-induced Ca^{2+} influx could explain in part its inhibition on the contraction evoked by 5-HT.

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SECTION IV. CHARACTERIZATION OF SEROTONERGIC RECEPTORS MEDIATING CONTRACTION OF THE OVINE UMBILICAL ARTERY[1]

Abstract

Responses to serotonergic agonists were studied in isolated umbilical arteries obtained from fetuses within 2 weeks of term. The order of potency of the agonists was determined to be 2,5-dimethoxy-4-methyl-amphetamine (DOM) > 5-HT > α-methyl-5-HT > 1-(3-chlorophenyl) piperazine (mCPP) = m-trifluoromethyl-phenylpiperazine (TFMPP) > 8-hydroxy-dipropylaminotetralin (8-OH-DPAT) > 2-methyl-5-HT > 1-(2-methoxyphenyl) piperazine (2-MPP). Variations in the sensitivity and potency of the agonists results primarily from the variation in the affinity for the 5-HT_2 receptor and less so in the efficacy. Alpha-methyl-5-HT was a full agonist compared to 5-HT. The others were partial agonists. The mean K_A values for 5-HT and DOM were 4.71 ± 0.62 x 10^{-7}M and 0.36 ± 0.04 x 10^{-7}M, respectively. Contractions to 5-HT and DOM were antagonized by ketanserin with pA_2 values being 9.4 and 9.1, respectively, suggesting that they act on the same receptor and that their responses are mediated by 5-HT_2 receptors. Contractile responses to 8-OH-DPAT, 2-methyl-5-HT and the phenylpiperazines (TFMPP and mCPP) were blocked by ketanserin (10^{-8}M), indicating that contractions produced by these agonists were mediated by 5-HT_2 receptors. There is a significant correlation between the dissociation constants of the

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[1]Submitted to J. Pharmacol. Exp. Ther. as a research paper by Lubo Zhang and Donald C. Dyer.

serotonergic agonists in producing vasoconstriction in the ovine umbilical artery and their affinity at 5-HT_2 binding sites in brain tissues while there is a negative correlation with the 5-HT_1 binding sites in brain tissues. No antagonism by 3-tropanyl-3,5-dichlorobenzoate (MDL 72222) of responses to 5-HT indicates that 5-HT_3 receptors are not present in this tissue.

Introduction

The first classification of 5-HT receptors comes from the work of Gaddum and Picarelli (1957) who demonstrated the existence of two pharmacologically distinct 5-HT receptors (D and M receptors) in the guinea-pig ileum. The D receptor is sensitive to dibenzyline (phenoxybenzamine) and mediates constriction of the smooth muscle. The M receptor is sensitive to morphine and mediates depolarization of the cholinergic nerves. Since phenoxybenzamine (dibenzyline) and morphine are not specific 5-HT receptor blocking drugs, they have no value as tools for the classification of 5-HT receptors. Recently, two classification schemes of 5-HT receptors have been proposed (Bradley et al., 1986; Peroutka and Snyder, 1979). Major advances in our knowledge have undoubtedly arisen as a result of new drug tools recently identified as 5-HT_2 receptor antagonist ketanserin (Leysen et al., 1981; Leysen et al., 1982; Van Nueten et al., 1981) and the 5-HT_3 receptor antagonist MDL 72222 (3-tropanyl-3, 5-dichlorobenzoate) (Fozard, 1984). The characterization of 5-HT receptors with the corresponding pharmacological responses has been investigated in various tissues,

including blood vessels. Vasoconstriction in many arteries has been found to be mediated by a receptor similar to the 5-HT_2 binding site (Van Nueten et al., 1981, 1984; Leysen et al., 1984; Maayani et al., 1984). The classification of 5-HT_1 receptors is somewhat less clear since selective antagonists have yet to be described.

We have shown that the hallucinogen 2,5-dimethoxy-4-methyl-amphetamine (DOM) is potentially harmful to the fetus in that it strongly activates uterine and umbilical vascular 5-HT receptors which produce vasoconstriction (Dyer et al., 1973; Dyer, 1983; Zhang and Dyer, 1988). The purpose of this study was to define the activity of 5-HT and DOM in terms of dissociation constants and to ascertain if 5-HT and DOM activate 5-HT_2 receptors in the ovine umbilical artery. By using specific competitive antagonists we were able to classify, in part, the subtype(s) of 5-HT receptors in the ovine umbilical artery.

Methods

Umbilical cords were obtained from mixed breed sheep within two weeks of term (145 days). The umbilical cord was sectioned at least 3 inches from the fetus. Helically cut umbilical arteries were prepared as previously described (Dyer, 1970). The arterial strips were suspended in 10-ml isolated organ baths, maintained at 37°C and bathed with a modified Krebs-Henseleit (Krebs') solution of the following composition (mM): NaCl, 115.21; KCl, 4.70; $CaCl_2$, 1.80; $MgSO_4$, 1.16; KH_2PO_4, 1.18; $NaHCO_3$, 22.14; dextrose, 7.88. Disodium ethylenediamine tetracetic acid (EDTA 0.03 mM) was added to suppress the oxidation of

amines. The Krebs' solution was oxygenated with a mixture of oxygen-carbon dioxide (95:5). The tissues were allowed to relax under 1g of tension for 90 min before initiating the experiment. Contractions were recorded isotonically. Concentration-response data were obtained by the cumulative additions of agonists in approximately one-half log increments (van Rossum, 1963).

Determination of agonist potency

Concentration-response data to serotonergic agonists were obtained after each tissue had been exposed to KCl (150mM) and this response to KCl was set as the 100% response. EC_{50} value for each agonist was recorded at the molar concentration where the curve intersected the 50% level of the response axis to that agonist. Relative potencies were calculated by comparing the molar concentration of agonists required to produce an equivalent percent contraction as that of KCl (150mM) at the 40% response.

Determination of agonist dissociation constants (K_A or K_p) and relative efficacy (e_r)

Because of the different characteristics of the various agonists, two procedures were used to ascertain their dissociation constants. For 5-HT, the K_A value was determined as described by Furchgott and Bursztyn (1967). Dibenamine was used to inactivate a fraction of the receptors. After fractional inactivation of the receptors by dibenamine (7.5×10^{-8}M, for 15 minutes), the bath fluid was changed 5 or 6 times over 30 minutes. The reciprocals of the concentrations of 5-HT before dibenamine treatment (1/[A]) were plotted against the reciprocals of the corresponding equieffective concentrations after treatment (1/[A']).

From the slope and intercept of the straight line fitting the points, the value for K_A and for the fraction of active receptors remaining (q) were calculated on the basis of the equation (Furchgott, 1966):

$$\frac{1}{[A]} = \frac{1-q}{qK_A} + \frac{1}{q[A']} \qquad (1)$$

according to which K_A equals (slope -1)/intercept, and q equals 1/slope.

For determining the dissociation constant (K_p) of a partial agonist the technique of Waud (1969) was inapplicable in the present study since the umbilical artery was found not to have a large spare receptor capacity for the full agonist, 5-HT [see Kenakin (1984) for a discussion of this requirement]. The procedure developed by Gero and Tallarida (1977) was, therefore, applied to DOM, phenylpiperazine derivatives (TFMPP, mCPP and 2-MPP), 2-methyl-5-HT and 8-OH-DPAT, which were partial agonists on the umbilical artery. Contractile responses produced by the partial agonist (P), DOM, phenylpiperazines, 2-methyl-5-HT and 8-OH-DPAT, were matched with those of the full agonist (A), 5-HT. The dissociation constants (K_p) for these partial agonists were obtained from the equation (Gero and Tallarida, 1977):

$$K_p = \frac{K_A\,(A_p - A_i) \cdot Pi}{(A_p + K_A) \cdot A_i} \qquad (2)$$

where K_A was the dissociation constant obtained for 5-HT [using the procedure of Furchgott and Bursztyn (1967) as described above], A_p was the concentration of the full agonist A (i.e., 5-HT) producing a response equal to the maximum of the partial agonist P (DOM, phenylpiperazines, 2-methyl-5-HT and 8-OH-DPAT) and A_i and P_i were any

other pair of concentrations giving a matching contractile response.

The relative affinities for the serotonergic agonists as compared to 5-HT were obtained by dividing the K_A value obtained for 5-HT by those (K_p) obtained for the other agonists.

To obtain the relative efficacies (e_r) for the serotonergic agonists as compared to 5-HT, the respective fractional occupation of receptors by each agonist for each concentration used in establishing the control concentration-response curves was calculated from the equation (Furchgott and Bursztyn, 1967).

$$\frac{[RA]}{[R_T]} = \frac{[A]}{[A] + K_A} \qquad (3)$$

Where [RA] is the concentration of the receptor-agonist complex, $[R_T]$ is the total concentration of receptors, [A] is any given concentration of agonist and K_A (or K_p) is the agonist dissociation constant. The contractile response data for each agonist were then replotted to show response as a function of log $[RA]/[R_T]$. The antilog of the distance between the two curves along the abscissa was taken as the ratio of the intrinsic efficacy of the second agonist to that of 5-HT (Furchgott and Bursztyn, 1967), and thus was a measure of e_r, the relative efficacy of the second agonist as compared to 5-HT.

In all experiments, cocaine (3×10^{-6}M) was added to block uptake mechanisms (Dyer, 1970) and iproniazid was used (0.36 mM) to block monoamine oxidase (MAO). Iproniazid was added for 40 minutes and then tissues were washed four times over 30 minutes with fresh Krebs' solution. Cocaine was added 15 minutes prior to adding the agonists.

Determination of apparent dissociation constants for antagonists

Ketanserin and MDL 72222 were used in a series of experiments to determine the subtypes of 5-HT receptors involved in contraction of the ovine umbilical artery. Initially a concentration-response curve was obtained to a specific agonist. The antagonists were then allowed to equilibrate for 1 hour with the tissue before repeating the concentration-response curve to the same agonist. The concentration ratio (CR) of the agonist (EC_{50} in the presence of antagonists/EC_{50} in the absence of antagonists) was determined. The time related shift of the agonist response curve was measured in a matched preparation not treated with antagonist. The concentration ratio (R_T) (EC_{50} at time t/EC_{50} at time 0) was determined from the two control agonist concentration response curves. The concentration ratio (CR) obtained from the antagonist treated tissue was then adjusted according to the following formula:

$$\text{Adjusted CR} = \frac{CR}{CR_T}$$

Cocaine and iproniazid were also used as described above.

Schild plots were employed to determine the pA_2 values for the antagonists. The adjusted CR (see above) obtained from the shifts in the agonist concentration-response curve by the different concentrations of the antagonist were utilized in the Schild equation (Arunlakshana and Schild, 1959):

$$\log (CR - 1) = \log [B] - \log K_B \qquad (4)$$

where [B] is the molar concentration of the antagonist and K_B is the dissociation constant of the antagonist. A linear regression performed

on the line generated by plotting log (CR -1) vs. -log [B] will have a slope of -1 if the antagonism is competitive. The intercept along the abscissa (i.e., when CR = 2) represents the negative log of the K_B for a competitive antagonist (i.e., pA_2). The mathematical calculations involved in determining a pA_2 value were performed using a computer program based on procedures outlined by Tallarida and Murray (1981).

Apparent dissociation constants (K_B) of ketanserin agonist 8-OH-DPAT, 2-methyl-5-HT, TFMPP and mCPP were determined for each tissue and at each concentration of antagonist, using the equation (Furchgott, 1972)

$$K_B = \frac{[B]}{CR - 1} \qquad (5)$$

where [B] is the concentration of antagonist and CR is the adjusted concentration ratio as discussed above.

Drugs

The following drugs were used: cocaine HCl; serotonin creatinine sulfate (Sigma Chemical Co., St. Louis, MO); R(-)-2,5-dimethoxy-4-methyl-amphetamine (National Institute of Drug Abuse, Rockville, MD); 8-hydroxy-dipropylaminotetralin (8-OH-DPAT), α-methyl-5-hydroxytryptamine (α-methyl-5-HT), 2-methyl-5-hydroxytryptamine (2-methyl-5-HT), m-trifluoromethyl-phenylpiperazine HCl (TFMPP), 1-(3-chlorophenyl) piperazine HCl (mCPP), 1-(2-methoxyphenyl) piperazine HCl (2-MPP), 3-tropanyl-3, 5-dichlorobenzoate (MDL 72222) (Res. Biochem. Inc., Natick, Massachusetts); Ketanserin tartrate (Janssen, Beerse, Belgium); iproniazid phosphate, (Hoffmann-LaRoche, Nutley, NJ); dibenamine HCl (Smith, Kline and French Lab., Philadelphia, PA); l-norepinephrine

bitartrate (Calbiochem Behring Corp., La Jolla, CA). Drugs were dissolved in saline, except for dibenamine and MDL 72222, which were dissolved in alcohol and diluted in saline just prior to use.

Data are expressed as mean ± SE; for each experiment, n refers to the number of fetal lambs from which vessels were taken. The Student's t-test was used for statistical analysis of the differences of means.

Results

Contractions of ovine umbilical artery by serotonergic agonists

Concentration-response curves to serotonergic agonists in the cord umbilical artery are illustrated in Fig. 1. The contractions were smooth and readily reproducible. In Table 1 the EC_{50} values and potency ratios are presented. The order of potencies of the agonists was determined to be DOM>5-HT>α-methyl-5-HT>mCPP = TFMPP>8-OH-DPAT>2-methyl-5-HT>2-MPP. DOM was 5 times more potent than 5-HT but only produced 78% of the maximal response to that of 5-HT. α-methyl-5-HT was slightly less potent than 5-HT with its maximal response being similar to that of 5-HT. 8-OH-DPAT, 2-methyl-5-HT and the phenylpiperazines, TFMPP, mCPP and 2-MPP only produced 32 to 61% of the maximum responses to that of 5-HT. TFMPP and mCPP were about 3.5 times less potent than 5-HT. 8-OH-DPAT was about 67 times less potent than 5-HT. 2-methyl-5-HT and 2-MPP were 250 and 500 times, respectively, less potent than 5-HT.

Agonist dissociation constants (K_A or K_p)

The response to 5-HT before and after exposing the tissues to

Fig. 1. Concentration-contractile response relationship for serotonergic agonists. Results are illustrated as the $\bar{x} \pm$ SE. of tissues from 3 to 8 animals and are expressed as percentage of the contraction obtained to 150mM KCl

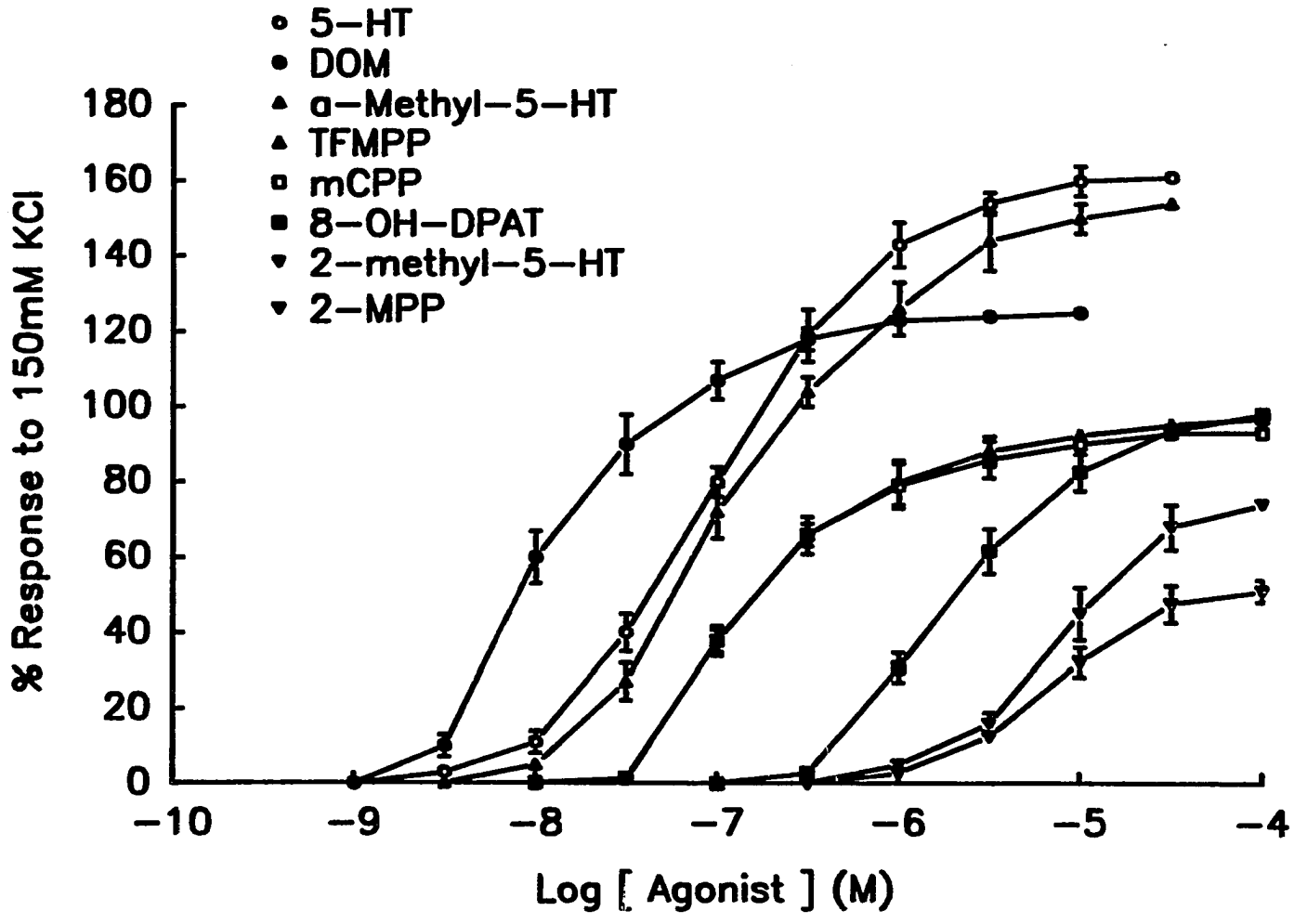

5–HT
DOM
α–Methyl–5–HT
TFMPP
mCPP
8–OH–DPAT
2–methyl–5–HT
2–MPP
% Response to 150mM KCl
180
160
140
120
100
80
60
40
20
0
–10
–9
–8
–7
–6
–5
–4
Log [Agonist] (M)

Table 1. Comparative properties of serotonergic agonists on the ovine umbilical artery

Agonist	N[a]	EC_{50}[b]	Relative Potency[c]	Approximate EC_{100}[d]	Maximum Response as a Percentage of the Response to 150mM KCl
		M (x 10^{-7})		M	%
5-HT	8	1.04 ± 0.27	1.000	3.0×10^{-5}	161
DOM	8	0.10 ± 0.02	5.000[e]	1.0×10^{-5}	126
α-Methyl-5-HT	7	1.25 ± 0.27	0.714[e]	3.0×10^{-5}	157
TFMPP	3	1.58 ± 0.25	0.280[e]	1.0×10^{-4}	96
mCPP	3	1.54 ± 0.19	0.280[e]	1.0×10^{-4}	93
8-OH-DPAT	4	19.95 ± 2.40	0.015[e]	1.0×10^{-4}	98
2-Methyl-5-HT	3	70.79 ± 9.10	0.004[e]	1.0×10^{-4}	74
2-MPP	3	79.43 ± 9.89	0.002[e]	1.0×10^{-4}	52

[a]N is the number of animals.
[b]EC_{50} is the effective concentration to produce 50% of the maximal response to the respective agonist.
[c]Relative potency was calculated at the EC_{40} (see Methods), 5-HT arbitrarily set at 1.
[d]EC_{100} is the effective concentration to produce the maximal response to the respective agonist.
[e]Significantly different from 1 ($P<0.05$).

dibenamine (7.5×10^{-8}M for 15 min) is shown in Fig. 2. Dibenamine reduced the maximal response to 5-HT about 45%. The inset (Fig. 2) illustrates a double reciprocal plot of equieffective concentrations of 5-HT before (1/[A]) and after (1/[A']) dibenamine treatment. The mean 5-HT dissociation constant (K_A) for the individual tissues was $4.71 \pm 0.62 \times 10^{-7}$M (Table 2). The dissociation constants (K_p) for the partial agonists DOM, 8-OH-DPAT, 2-methyl-5-HT, TFMPP, mCPP and 2-MPP are presented in Table 2. The relative affinities of the agonists studied as compared to 5-HT were calculated by dividing the estimated K_A of 5-HT by K_p values of the other agonists (Table 2). The affinity of DOM was, on the average, 13 times that of 5-HT ($P<0.05$). TFMPP and mCPP possessed similar affinities to that of 5-HT. The relative affinities of the other three agonists were lower, ranging from 9% in the case of 8-OH-DPAT to 4% in the case of 2-methyl-5-HT and 2-MPP when compared to 5-HT (Table 2).

Relative efficacy (e_r) of agonists

The average dissociation constants (K_A or K_p) values of 5-HT and other agonists were used in Equation 3 (see "Methods") to calculate the fraction of receptors occupied ($[RA]/[R_T]$) at each concentration employed in order to obtain the complete concentration-response curves for the agonists. The results of replotting relative response against log $[RA]/[R_T]$ are shown in Fig. 3. The curve for 5-HT indicates that one-half the maximal response is obtained when about 18% of receptors are occupied by 5-HT, and that 90% of the maximum is obtained when about 69% of receptors are occupied. The relative efficacies of DOM, 8-OH-DPAT, 2-methyl-5-HT and the phenylpiperazines to 5-HT were obtained as

Fig. 2. Effect of dibenamine on 5-HT-elicited contractions of ovine umbilical artery. Contractions were obtained before (open circles) and after (closed circles) exposure to 7.5×10^{-8}M dibenamine for 15 min. Dibenamine was washed out of the tissue before obtaining the second concentration-response to 5-HT. Each point represents the $\bar{x} \pm$ SE. of tissues from 8 animals. Inset: Plot of the reciprocals of equieffective concentrations of 5-HT before (1/[A]) and after (1/[A']) dibenamine treatment (See Methods)

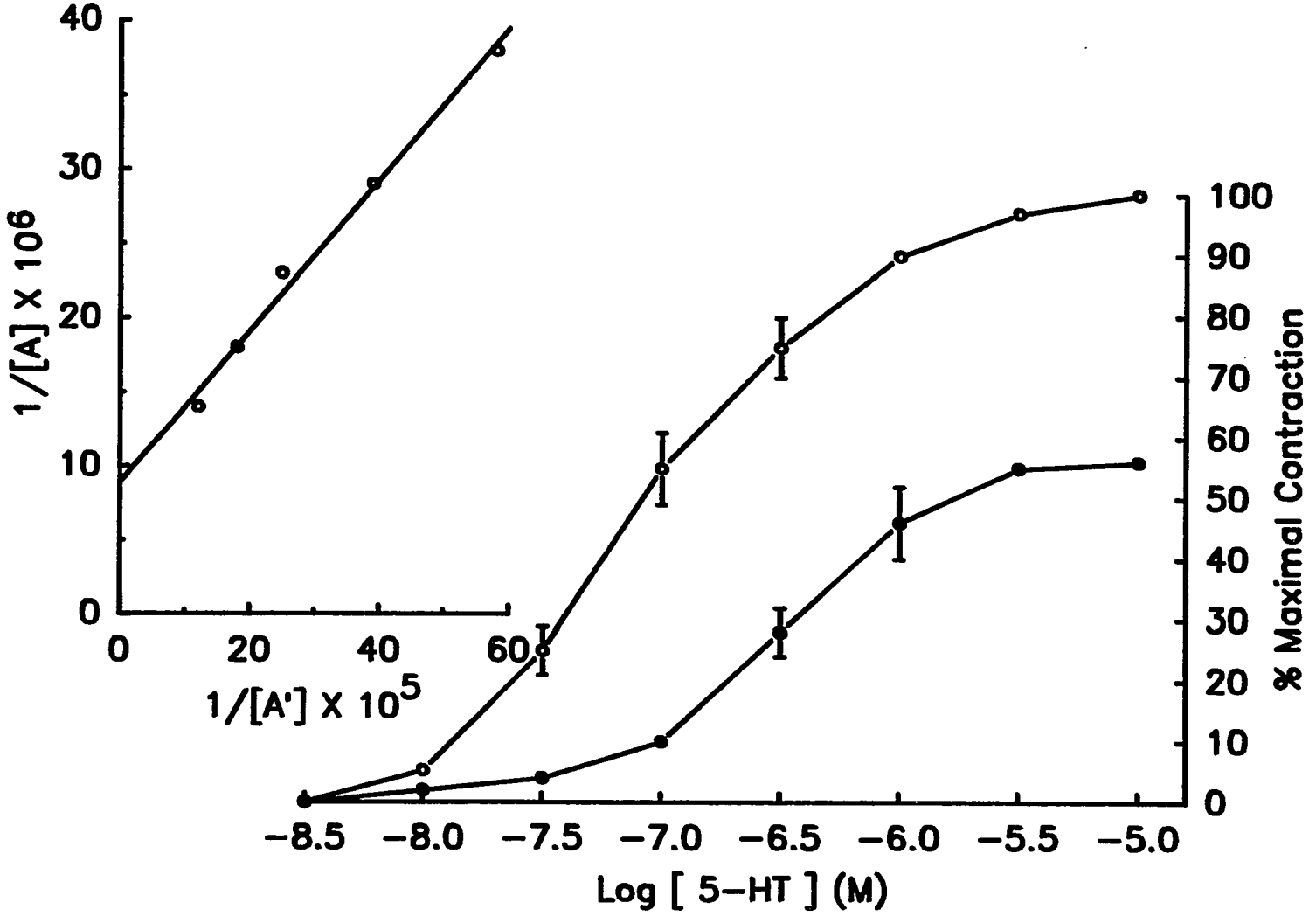
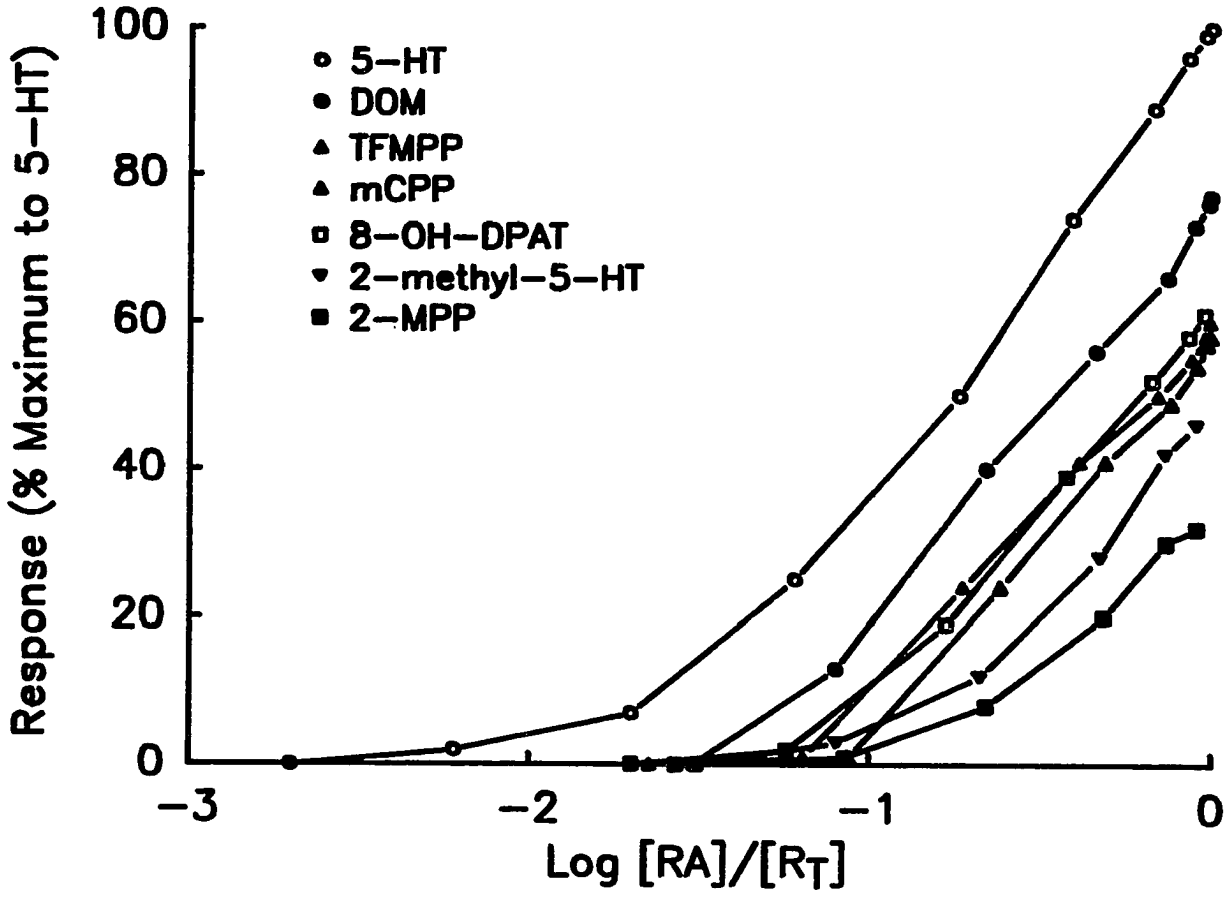

40
30
20
10
0
1/[A] X 10^6
0
20
40
60
1/[A'] X 10^5
100
90
80
70
60
50
40
30
20
10
0
% Maximal Contraction
−8.5
−8.0
−7.5
−7.0
−6.5
−6.0
−5.5
−5.0
Log [5−HT] (M)

Table 2. Comparison of dissociation constant (K_A or K_p), relative efficacy (e_r) and relative affinity for the agonists acting on the serotonergic receptors in the ovine fetal umbilical artery

Agonist	N[a]	Dissociation Constant	Relative Efficacy[b]	Relative Affinity[c]
		M ($\times 10^{-7}$)		
5-HT	8	4.71 ± 0.62	1.00	1.00
DOM	8	0.36 ± 0.04	0.64[d]	13.08[d]
TFMPP	3	4.34 ± 1.14	0.41[d]	1.08
mCPP	3	3.15 ± 0.97	0.32[d]	1.49
8-OH-DPAT	4	49.81 ± 12.11	0.41[d]	0.09[d]
2-Methyl-5-HT	3	113.16 ± 37.27	0.25[d]	0.04[d]
2-MPP	3	107.14 ± 23.48	0.15[d]	0.04[d]

[a]N is the number of animals.
[b,c]5-HT is arbitrarily set at 1.
[d]Significantly different from 1 ($P<0.05$).

Fig. 3. Contractions to serotonergic agonists plotted as a function of log $[RA]/[R_T]$ in the ovine umbilical artery. Receptor occupancy ($[RA]/[R_T]$) at a given concentration of agonist was calculated as described under "Methods" using the mean dissociation constant obtained for each agonist, as given in Table 2. Contractile responses were taken from the results illustrated in Figure 1


100
80
60
40
20
0
−3
−2
−1
0
Response (% Maximum to 5−HT)
Log [RA]/[RT]
5−HT
DOM
TFMPP
mCPP
8−OH−DPAT
2−methyl−5−HT
2−MPP

the antilog of the distance between the respective agonist and 5-HT along the log $[RA]/[R_T]$ axis. The mean values were presented in Table 2.

Correlations between sensitivity and relative potency of agonists and their affinity and efficacy

There is a significant linear correlation between $_pD_2$ (-log EC_{50}) of the individual agonists and their pK_A (-log K_A) where $r = 0.993$, $P < 0.001$ (Fig. 4). The slope of the regression line was 0.86, which was not significantly different from unity. Fig. 5 (left panel) shows that there is a significant correlation between relative potency (RP) and the relative affinity (RA) of the agonists where $r = 0.974$ ($P < 0.001$). The slope of the regression line was 0.72 which was significantly different from unity. The correlation between relative potency and the relative efficacy (RE) of the agonists was also significant with $r = 0.829$ ($P < 0.05$) (Fig. 5, right panel). The slope of the regression line was 0.21 which was significantly different from unity.

Competitive antagonists

Ketanserin competitively inhibited responses to DOM as demonstrated by producing parallel shifts in the log concentration-response curves to the right (Fig. 6). The maximal response was not affected by ketanserin at the highest concentration used (10^{-7}M). Schild plot for ketanserin vs. DOM yields a straight line with the slope being -0.91, which was not significantly different from unity. The concentration-response curves for 5-HT were shifted to the right by ketanserin similarly as those for DOM. The pA_2 values for ketanserin vs. DOM and 5-HT were 9.1 and 9.4, respectively. Contractile responses to 8-OH-DPAT, 2-methyl-5-HT, TFMPP

Fig. 4. Relationship between pK_A (-log K_A) and pD_2 (-log EC_{50}) of serotonergic agonists in the ovine umbilical artery. EC_{50} value for each agonist was recorded at the molar concentration where the curve intersected the 50% level of the response axis to that agonist. Correlation coefficient $r = 0.993$, $P < 0.001$. Slope is 0.86 which is not significantly different from unity

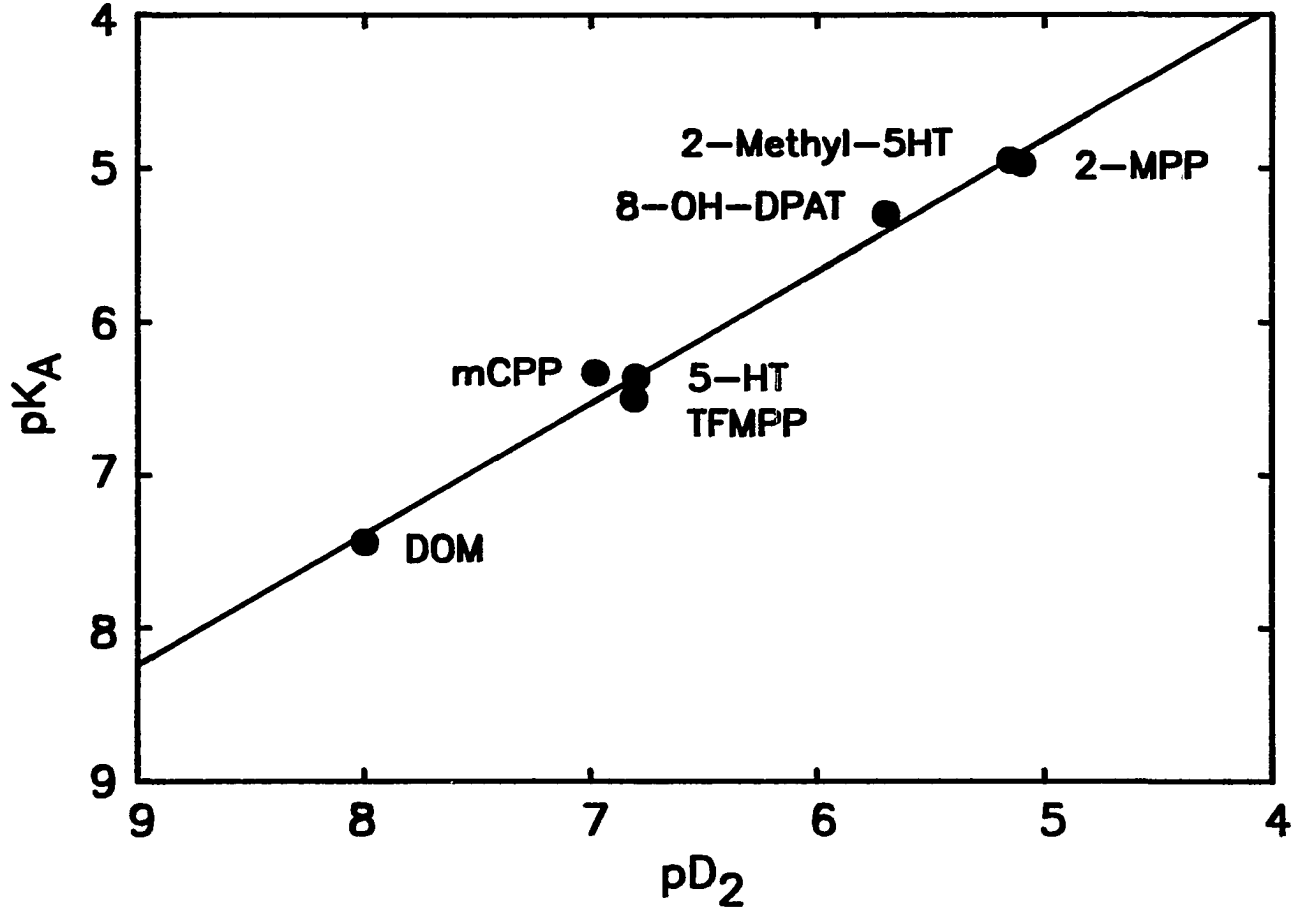

4
5
6
7
8
9
9
8
7
6
5
4
pK_A
pD_2
2-Methyl-5HT
2-MPP
8-OH-DPAT
mCPP
5-HT
TFMPP
DOM

Fig. 5. Relationship between the relative potency (RP) and the relative affinity (RA) (left panel) and the relative efficacy (RE) (right panel) of the sertonergic agonists in the ovine umbilical artery. The relative potency, relative affinity and relative efficacy were determined as described in Methods. For the relationship between RP and RA, $r = 0.974$, $P < 0.001$; slope = 0.72. For the relationship between RP and RE, $r = 0.829$, $P < 0.05$; slope = 0.21

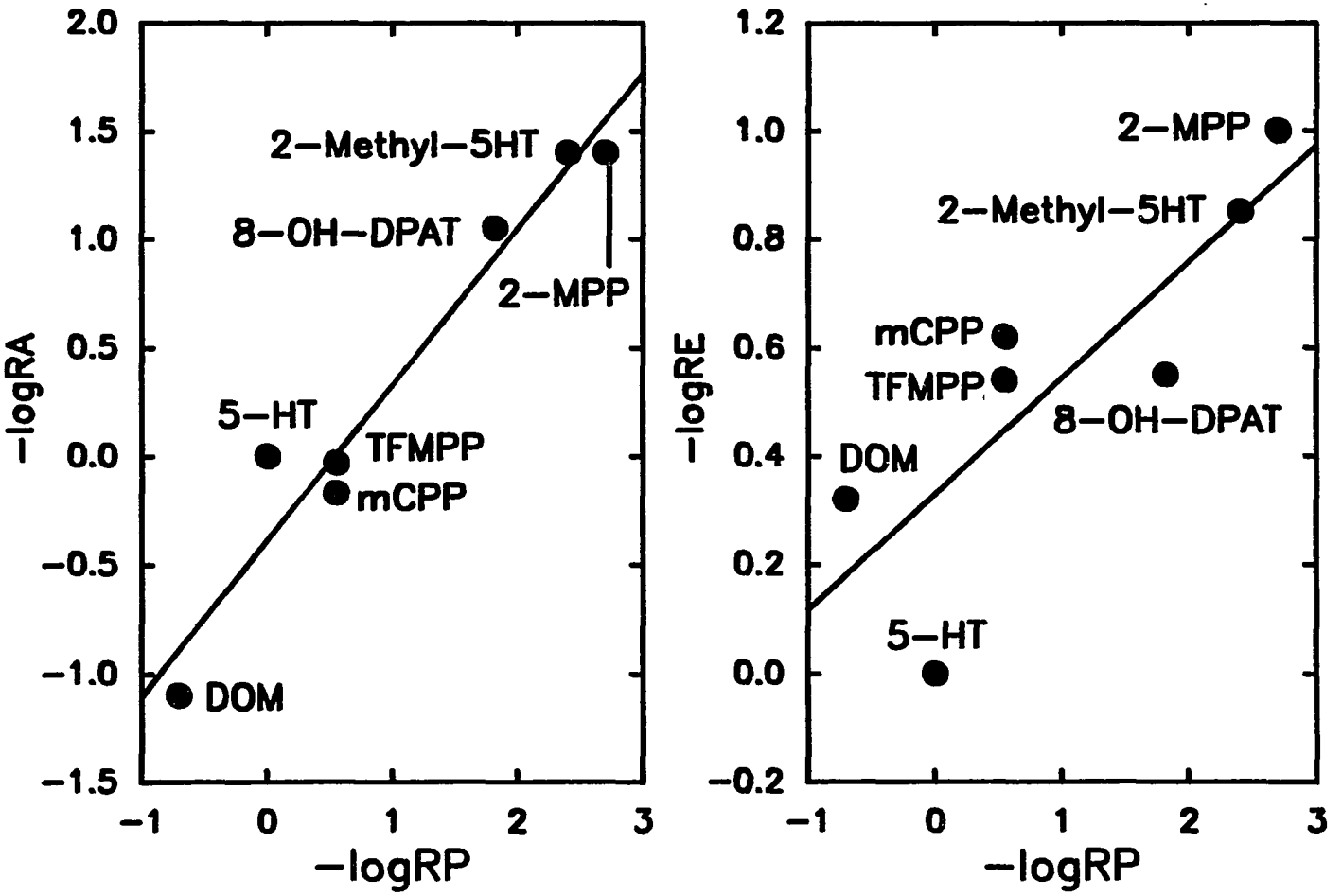
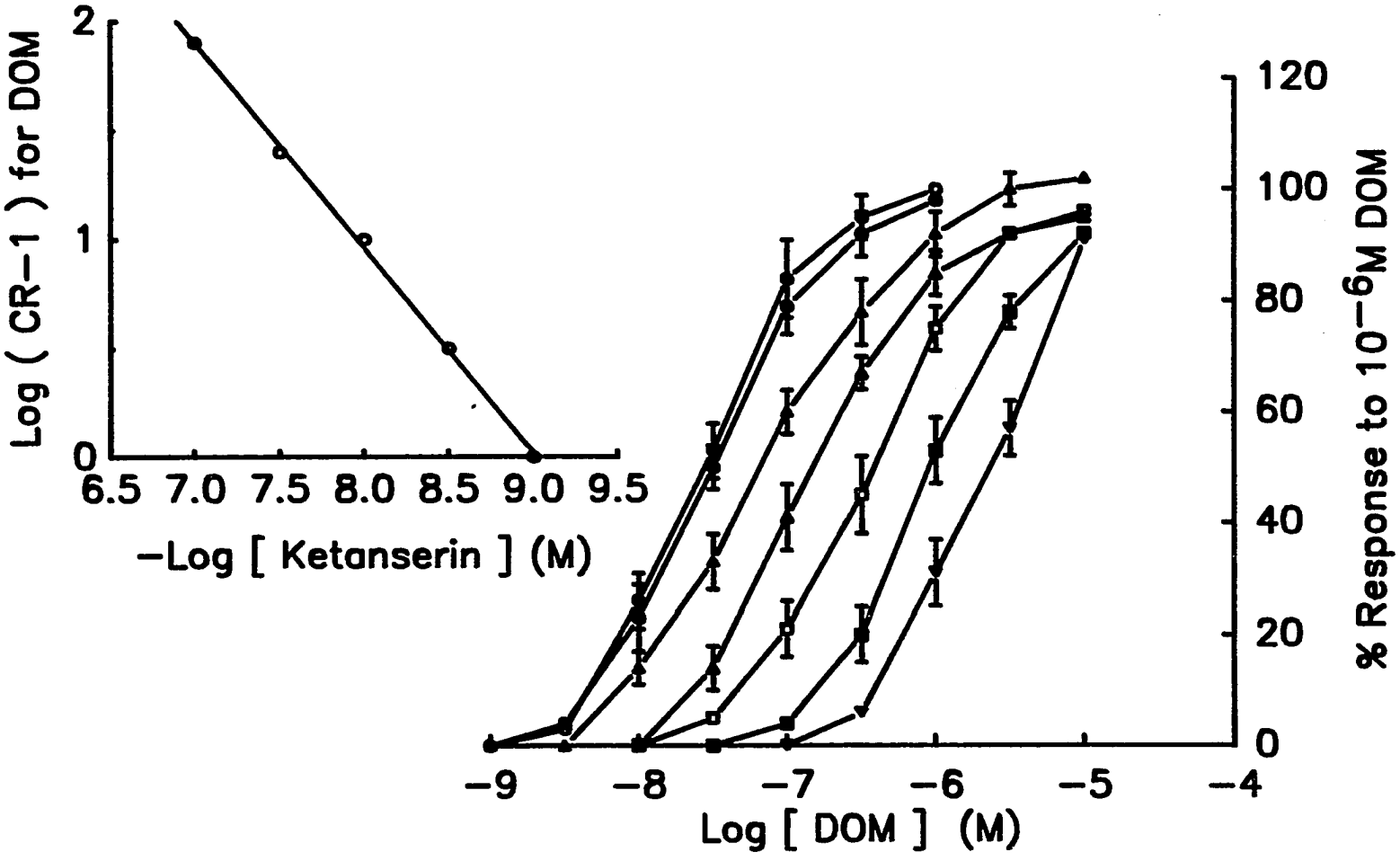

2.0
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5
−logRA
2−Methyl−5HT
8−OH−DPAT
2−MPP
5−HT
TFMPP
mCPP
DOM
−1
0
1
2
3
−logRP
1.2
1.0
0.8
0.6
0.4
0.2
0.0
−0.2
−logRE
2−MPP
2−Methyl−5HT
mCPP
TFMPP
8−OH−DPAT
DOM
5−HT
−1
0
1
2
3
−logRP

Fig. 6. Cumulative concentration-response curves for DOM obtained on ovine umbilical artery in the absence and presence of various concentrations of ketanserin (open circle: DOM control; closed circle: time control; open triangle, 10^{-9}M; closed triangle, 3 x 10^{-9}M; open square, 10^{-8}M; closed square, 3 x 10^{-8}M; reversed open triangle, 10^{-7}M). Each point represents the $\bar{x}$ ± S.E. of tissues from 8 animals and is expressed as a percentage of the contraction obtained to 10^{-6}M DOM in control experiments. Inset: Schild plot for determination of the pA_2 for ketanserin vs. DOM. The intercept on the abscissa gives the pA_2 value (9.10). The slope (-0.91) of the fitted regression line was not significantly different from unity


Log (CR−1) for DOM
2
1
0
6.5 7.0 7.5 8.0 8.5 9.0 9.5
−Log [Ketanserin] (M)
120
100
80
60
40
20
0
% Response to 10^{-6}M DOM
−9 −8 −7 −6 −5 −4
Log [DOM] (M)

and mCPP were also effectively blocked by ketanserin (10^{-8}M) and the concentration-responses curves for these agonists were shifted in a parallel manner to the right without affecting the maximum response. The dissociation constants (K_B) of ketanserin vs. 8-OH-DPAT, 2-methyl-5-HT, TFMPP and mCPP are presented in Table 3. No antagonism by MDL 72222 (10^{-8}M to 10^{-6}M) of contractions to 5-HT was observed (Fig. 7).

Discussion

The validity of the determination of K_A value carried out for 5-HT depends on the acceptance of the theoretical assumptions of receptor-agonist interactions following fractional irreversible receptor inactivation as outlined by Furchgott (1966). The K_A value for 5-HT (0.47μM) is not significantly different from that (0.37μM) determined in the ovine uterine artery (Zhang and Dyer, 1989), implying that the same type of 5-HT receptors are involved in the contraction to 5-HT in these two vessels. The difference in dissociation constants determined for 5-HT and DOM in this study indicates there are differences in the affinity of these two agonists for 5-HT receptors. By comparing the two dissociation constants, the affinity of DOM was found to be 13 times that of 5-HT. Our results are comparable to those previously reported in binding studies in brain tissue (Glennon, 1987). It has been shown that 5-HT displayed a high affinity (K_i = 1-10nM) for 5-HT_1 binding sites but a relatively low affinity (K_i = 0.4 - 1 μM) for 5-HT_2 binding sites. DOM was identified as a 5-HT_2 agonist with a relatively high affinity (K_i = 0.1 μM) for 5-HT_2 binding sites (Glennon, 1987).

Table 3. Dissociation constants (K_B) for ketanserin against the agonists acting on the serotonergic receptors in ovine fetal umbilical artery

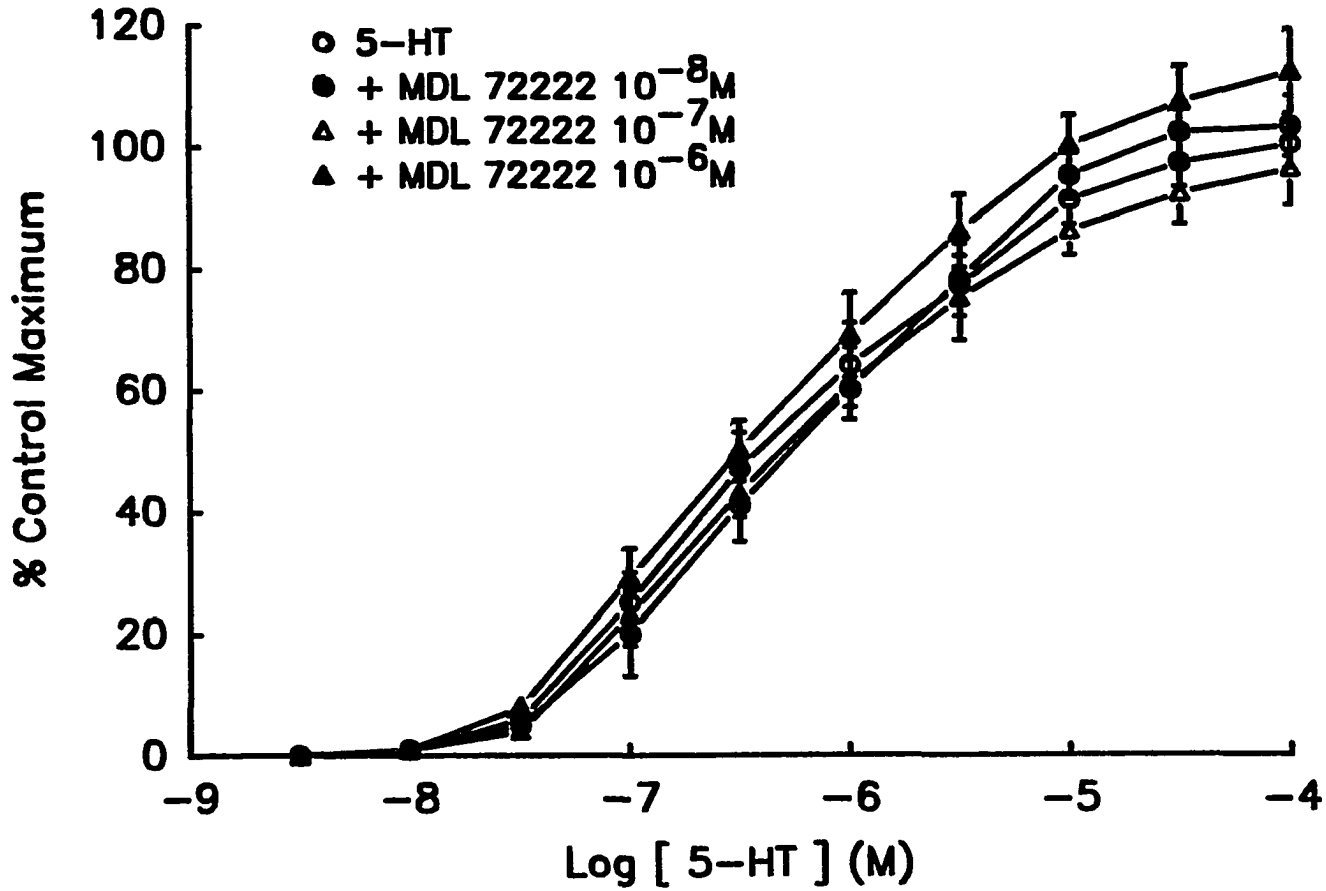
Agonist	N[a]	K_B
		nM
5-HT	8	0.40 ± 0.05[b]
DOM	8	0.79 ± 0.10[b]
TFMPP	4	1.01 ± 0.31[c]
mCPP	4	0.38 ± 0.12[c]
8-OH-DPAT	4	0.29 ± 0.09[c]
2-Methyl-5HT	3	0.49 ± 0.17[c]

[a]N is the number of animals.

[b]K_B values are obtained from the pA_2 values according to the relationship $pA_2 = -\log K_B$.

[c]K_B values are calculated for each tissue at ketanserin 3 X 10^{-9}M according to the equation $K_B = [\text{antagonist}]/CR-1$.

Fig. 7. Cumulative concentration-response curves for 5-HT obtained on ovine umbilicl artery in teh absence and presence of various concentrations of MDL 72222. Each point represents the average of tissues from 4 animals and is expressed as a percentage of the contraction obtained to 150mM KCl


5–HT
+ MDL 72222 10⁻⁸M
+ MDL 72222 10⁻⁷M
+ MDL 72222 10⁻⁶M
% Control Maximum
120
100
80
60
40
20
0
−9
−8
−7
−6
−5
−4
Log [5–HT] (M)

Differences in agonists' potencies in a biologic system could result from differences in their affinities for the receptors and/or their relative efficacies (see discussion by Kenakin, 1984). In the present study, there was a significant linear correlation between agonist potency and affinity ($r = 0.974$, $P < 0.001$) and relative efficacy ($r = 0.829$, $P < 0.05$). The slopes of the regression lines for the potency vs. the affinity and the potency vs. efficacy were 0.72 and 0.21, respectively. This implies that about 70% of the variation in the potency of the serotonergic agonists in producing vasoconstriction in the ovine umbilical artery is due to variation in the affinity of the agonists for the 5-HT_2 receptors. The variation in the efficacy of the agonists in this study contributes about 20% of the variation in the potency of the agonists. There is an excellent linear correlation ($r = 0.993$, $P < 0.01$) between sensitivity and the affinity (pD_2 versus pK_A) of serotonergic agonists. The slope of the regression line relating affinity to sensitivity was 0.86, which was not significantly different from unity. This suggests that the differences in the agonist's sensitivity in this study could be explained by the variation in the affinity alone. In the recent review article, Bevan et al. (1989) discussed the relationship between the receptor affinity and the agonist sensitivity in different vessels. In rabbit arteries, the slope of the regression line relating norepinephrine (NE) affinity for adrenergic receptors to its sensitivity was 0.90 (Oriowo et al., 1989). They suggested that differences in sensitivity of the rabbit arteries to NE was caused by variation in the receptor affinity. However, in rat arteries, variation in receptor affinity for NE only counted for 60% of

the variation in its sensitivity. The other 40% variation in NE sensitivity came from the variation in its receptor reserve (Oriowo et al., 1989).

In comparison to 5-HT, α-methyl-5-HT behaved as a full agonist while DOM and the other five agonists were partial agonists. The lower efficacies (e_r) of DOM and the other agonists when compared to 5-HT supports their classification as partial agonists in this tissue. It is interesting to note that the affinity of DOM did not correspond with its efficacy. This observation provides a striking example of the pharmacological principle that affinity and efficacy are separate and distinct properties possessed by agonists. The classification of DOM as a partial agonist is in an agreement with the findings by Dyer et al. (1973) and Sanders-Bush et al. (1988), but not with our recent finding in the uterine artery in which DOM was a full agonist (Zhang and Dyer, 1989). The reason for these differences of DOM is not quite clear.

There is little, if any, receptor reserve in this tissue, since nearly all the receptors need to be occupied by 5-HT to produce a maximal response. This is in accord with our recent findings in the ovine uterine artery (Zhang and Dyer, 1989). Evidence supporting a lack of spare receptors for 5-HT in other tissues was also obtained in canine basilar artery (Taylor et al., 1985) rat fundic strips (Clineschmidt, 1985) and rat jugular vein and uterus (Cohen et al., 1986).

In a previous study, cinanserin competitively blocked contractile responses to 5-HT and DOM in the ovine umbilical artery and produced similar pA_2 values for 5-HT (8.7) and DOM (8.5) (Dyer, 1983). Ketanserin has been documented to be a potent and selective 5-HT_2

receptor antagonist (Leysen et al., 1981; Leysen et al., 1982; Van Nueten et al., 1981). It possesses a high affinity (K_i < 1nM) for 5-HT_2 binding sites in the brain tissue (Glennon, 1987). In this study, a series of concentrations (10^{-9} to 10^{-7}M) of ketanserin effectively inhibited contractile responses to 5-HT and DOM and shifted the concentration-response curves to the right in a parallel manner without affecting the maximum response, consistent with competitive inhibition. This was supported by Schild plots whose slopes were not significantly different from unity. The pA_2 values of ketanserin against 5-HT (9.4) and DOM (9.1) in the present study are somewhat higher than those (8.56 and 8.33, respectively) determined previously in the ovine uterine artery (Zhang and Dyer, 1989) but are comparable to those determined on other blood vessels (8.1 to 9.7) (Cohen et al., 1983; Cohen, 1986; Humphrey, 1984; Van Nueten et al., 1982; Van Nueten et al., 1981). This suggests that the contractile responses produced by 5-HT and DOM in the ovine umbilical artery are mediated by 5-HT_2 receptors. The contractions produced by 8-OH-DPAT, 2-methyl-5-HT, TFMPP and mCPP were also blocked by ketanserin with the dissociation constants (Table 3) similar to those for 5-HT and DOM. This provides further evidence that these agonists are producing contractions via 5-HT_2 receptors in the ovine umbilical artery. The finding that DOM acts on 5-HT_2 receptors is in accord with the previous findings by us (Dyer, 1983; Zhang and Dyer, 1989) and those reported by Glennon and co-workers in the central nervous system (1983, 1984).

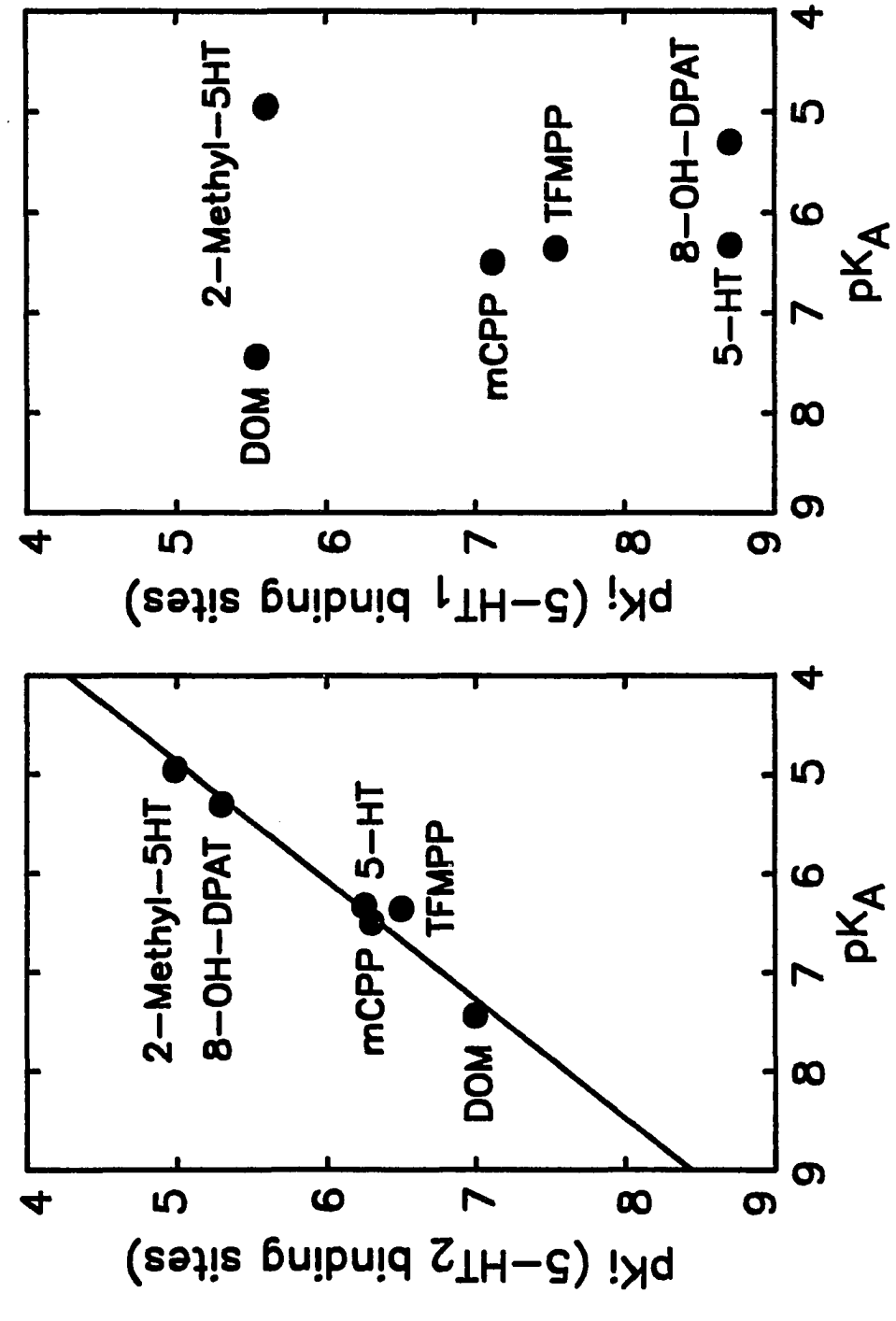
The finding of great interest is that the dissociation constants of the serotonergic agonists in producing vasoconstriction of the umbilical

artery is significantly correlated to their affinities at the 5-HT_2 binding sites in the CNS obtained by other workers (Fig. 8, left panel) but fails to correlate with those at the 5-HT_1 binding sites (Fig. 8, right panel). This provides good evidence that these serotonergic agonists produce vasoconstriction in the ovine umbilical artery by acting on 5-HT_2 reception and that these receptors are the same as those in the CNS.

8-OH-DPAT strongly binds to the 5-HT_{1A} binding site (K_i = 2nM) and weakly at the 5-HT_2 binding sites (K_i = 5500 to 7100 nM) in brain tissue (Glennon, 1987). It has been shown that 5-HT_{1A} receptors were linked to contraction in the canine basilar artery (Peroutka et al., 1986; Taylor et al., 1987). However, contractile responses to 8-OH-DPAT in the present study were unlikely mediated by 5-HT_{1A} receptors because ketanserin effectively blocked the contraction produced by 8-OH-DPAT. The dissociation constant of ketanserin vs. 8-OH-DPAT (0.29 nM) was not significantly different from that of ketanserin vs. 5-HT (0.40nM) in this study. This suggests that the contraction produced by 8-OH-DPAT in the ovine umbilical artery was mediated by 5-HT_2 receptors. This is in agreement with our recent finding in the ovine uterine artery (Zhang and Dyer, submitted for publication). The dissociation constant (4981 nM) of 8-OH-DPAT in this study was comparable to that at 5-HT_2 binding sites in brain tissue (5500 to 7100 nM) (Glennon, 1987) but was much higher than that at 5-HT_{1A} binding sites in brain tissue (2nM) Glennon, 1987).

Three phenylpiperazines used in this study, TFMPP, mCPP and 2-MPP, have been identified as ligands for 5-HT_{1B} binding sites in the rat brain (Sills et al., 1984; Fuller et al., 1980). The K_i values of TFMPP

Fig. 8. Correlation between the dissociation constant of the agonists ($pK_A = -\log K_a$) to produce the vasoconstriction in the ovine umbilical artery and their binding affinities ($pK_i = -\log K_i$) for 5-HT_2 binding sites in brain tissue (labelled with [^{3}H] ketanserin; 5-HT and DOM, Glennon, 1987; TFMPP and 8-OH-DPAT, Taylor et al., 1985; mCPP, Martin and Sanders-Bush, 1982; 2-methyl-5-HT, Engel et al., 1986) or 5-HT_1 binding sites (5-HT and DOM, labelled with [^{3}H]-5-HT, Glennon, 1987; TFMPP and mCPP labelled with [^{3}H]-ICYP, Glennon, 1987; 8-OH-DPAT, labelled with [^{3}H]-8-OH-DPAT, Glennon, 1987; 2-methyl-5-HT, labelled with [^{3}H]-8-OH-DPAT, Engel et al., 1986). The correlation coefficient of pK_A in this study and pK_i for 5-HT_2 and 5-HT_1 binding sites in the CNS are 0.983 ($P < 0.001$) and -0.187, respectively


pKi (5-HT2 binding sites)
pKA
2-Methyl-5HT
8-OH-DPAT
mCPP
5-HT
TFMPP
DOM
pKi (5-HT1 binding sites)
pKA
DOM
2-Methyl-5HT
mCPP
TFMPP
5-HT
8-OH-DPAT

and mCPP at 5-HT_{1B} sites were 30nM and 75nM, respectively (Glennon, 1987). However, the selectivity for 5-HT_1 vs 5-HT_2 binding sites was not high. TFMPP and mCPP possess only 3- to 18- fold selectivity for 5-HT_1 vs. 5-HT_2 sites (Glennon, 1987; Martin and Sanders-bush, 1982). 2-MPP possesses a 100-fold selectivity for 5-HT_1 vs. 5-HT_2 sites with an affinity for 5-HT_1 sites comparable to that of TFMPP (Glennon, 1987). TFMPP and mCPP are generally thought to be agonists at central 5-HT receptors which activate pre-synaptic autoreceptors on serotonin neurons, thereby modulating the neurogenic release of serotonin (Martin and Sanders-Bush, 1982). In the present study, these phenylpiperazines were demonstrated to be partial agonists on the ovine umbilical artery. Contractile responses to these phenylpiperazines were effectively blocked by ketanserin with the dissociation constants (Table 3) which were similar to that for ketanserin vs. 5-HT. This suggests that contractile responses to these phenylpiperazines on the ovine umbilical artery are mediated by 5-HT_2 receptors. In our recent study in ovine uterine artery, we demonstrated that TFMPP and mCPP were weak partial agonists and 2-MPP lacked agonist activity (Zhang and Dyer, submitted for publication). Instead, these three phenylpiperazines blocked contractions produced by 5-HT in the ovine uterine artery. The dissociation constants (K_B) of TFMPP (0.22 μM) and mCPP (0.13 μM) vs. 5-HT in the ovine uterine artery were comparable to those (K_p) of TFMPP (0.43 μM) and mCPP (0.31 μM) as partial agonists in the ovine umbilical artery. This suggests that the same type of 5-HT receptors was interacted by the phenylpiperazines in both vessels. Cohen et al. (1983) also showed that TFMPP and mCPP had a very weak agonist activity

in the rat jugular vein and could competitively antagonize 5-HT-induced contractions. The much lower agonist activity of the phenylpiperazines in the ovine uterine artery and in the rat jugular vein than in the ovine umbilical artery may be due to the differences in their relative efficacies and/or to differences in the efficiency of stimulus-response coupling in the different vessels (see discussion by Kenakin, 1984).

MDL 72222 is a selective and potent antagonist of 5-HT_3 receptors such as those mediating excitation of postganglionic sympathetic neurons (Fozard, 1984). MDL 72222 (10^{-8} to 10^{-6}M) did not inhibit contractions of the umbilical artery to 5-HT, providing a strong evidence that 5-HT_3 receptors are not present in the ovine umbilical artery. This is similar to our previous observation in the ovine uterine artery (Zhang and Dyer, 1989). Contractions to 2-methyl-5-HT, a selective 5-HT_3 receptor agonist (Bradley et al., 1986), were not blocked by MDL 72222 (10^{-6}M) but were effectively blocked by ketanserin (10^{-8}M). The dissociation constant (K_B) of ketanserin vs. 2-methyl-5-HT (0.49 nM) was similar to that of ketanserin vs. 5-HT (0.40nM), indicating that contractions produced to 2-methyl-5-HT were mediated by 5-HT_2 receptors in the ovine umbilical artery.

In summary, 5-HT and DOM are potent agonists and produce contractions via 5-HT_2 receptors in the ovine umbilical artery. DOM is 5 times more potent than 5-HT but only produces 78% of the 5-HT maximum contraction. Variations in the sensitivity and potency of the serotonergic agonists in this study result primarily from the variation in the affinity for the 5-HT_2 receptor. Vasoconstrictions produced by 8-OH-DPAT, 2-methyl-5-HT, TFMPP and mCPP were effectively antagonized by

ketanserin, suggesting that the constrictions were mediated by 5-HT_2 receptors. The highly significant correlation between the affinity of the agonists in producing vasoconstriction in the ovine umbilical artery with their affinities at 5-HT_2 binding sites in brain tissue supports the conclusion that 5-HT_2 receptors mediate the vasoconstriction in the ovine umbilical artery and they are similar to those 5-HT_2 receptors in the CNS. 5-HT_3 receptors were not present in the ovine umbilical artery.

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SECTION V. RECEPTOR MECHANISMS FOR 5-HYDROXYTRYPTAMINE (5-HT) IN THE ISOLATED OVINE UMBILICAL VEIN[1]

Abstract

5-hydroxytryptamine (5-HT) and 2,5-dimethoxy-4-methyl-amphetamine (DOM) produced a concentration-dependant contraction in isolated umbilical veins obtained from fetal lambs within 2 weeks of term. Contractions to 5-HT were antagonized by ketanserin, mianserin and methiothepin with the dissociation constants (K_B) being 2.17 ± 0.36 nM, 1.37 ± 0.55 nM and 1.98 ± 0.48 nM, respectively. The order of potency of serotonergic agonists in this tissue was: DOM > 5-HT > α-methyl-5-HT > 1-(3-chlorophenyl) piperazine (mCPP) > m-trifluoromethyl-phenylpiperazine (TFMPP) > 8-hydroxy-dipropylaminotetralin (8-OH-DPAT) = 2-methyl-5-HT. α-Methyl-5-HT was a full agonist compared to 5-HT. DOM possessed greater affinity but less efficacy than that of 5-HT. The affinities and efficacies of the other agonists studied were lower than those of 5-HT. Variation in the sensitivity and potency of agonists is primarily due to variations in their affinity for 5-HT receptors. Assessment of receptor occupancy vs. functional response demonstrated very little, if any, receptor reserve for 5-HT receptors in this tissue. Contractile responses to DOM, 8-OH-DPAT, mCPP and 2-methyl-5-HT were effectively blocked by ketanserin. The dissociation constants (K_B) of ketanserin against these agonists were as follows: DOM, 2.78 ± 0.85 nM;

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[1]Submitted to Eur. J. Pharmacol. as a research paper by Lubo Zhang and Donald C. Dyer.

8-OH-DPAT, 3.47 ± 1.12 nM; mCPP, 1.45 ± 0.51 nM; 2-methyl-5-HT, 1.99 ± 0.74 nM. The dissociation constant of MDL 72222 (3-tropanyl-3,5-dichlorobenzoate) vs. 5-HT was 13833 nM. No antagonism by prazosin (10^{-7}M) or yohimbine (10^{-7}M) of the responses to 5-HT was observed. These results indicate that 5-HT_2 receptors are present in the ovine umbilical vein. 5-HT_3 receptors were not present in this tissue. Activation of α-adrenergic receptors was not involved in the contractions to 5-HT.

Introduction

5-Hydroxytryptamine (5-HT) has been demonstrated to be a potent vasoconstrictor of ovine (Dyer, 1970) and human umbilical blood vessels (Gant and Dyer, 1971; Altura et al., 1972; Nair and Dyer, 1973). However, the receptor mechanisms involved in 5-HT induced vasoconstriction in the umbilical vein remain to be elucidated. Major advances in 5-HT receptor classification have become possible as a result of the availability of new drug antagonists such as ketanserin for the 5-HT_2 receptor and MDL 72222 (3-tropanyl-3,5-dichlorobenzoate) for the 5-HT_3 receptor. The classification of 5-HT_1 receptors in tissues is somewhat complicated since at least four subtypes have been proposed and highly selective antagonists are not available (Heuring and Peroutka, 1987; Pazos et al., 1984; Pedigo et al., 1981). Functional correlates of the various 5-HT_1 binding sites have been proposed (Peroutka, 1984) but are more speculative.

We demonstrated that 5-HT is a potent agonist mediating vasoconstriction in ovine uterine and umbilical arteries by acting on

$5HT_2$ receptors (Zhang and Dyer, 1989a, 1989b). The present study was conducted to characterize 5-HT receptor subtypes in ovine umbilical vein. The potency order of a series of serotonergic agonists was determined. The apparent dissociation constants were obtained for 5-HT and several competitive antagonists of 5-HT receptors. The characteristics of the serotonergic receptors in the umbilical vein are compared and related to the current receptor classification schemes.

Methods

Adult pregnant mixed breed sheep within two weeks of term (145 days) were anesthetized with pentobarbital and then exsanguinated. The pentobarbital was administered via the external jugular vein. An incision in the abdomen was made and the uterus exposed. Another incision was made in the uterus and the fetus delivered. The umbilical cord was sectioned at least 3 inches from the fetus. Helically cut umbilical veins were prepared as previously described (Dyer, 1970). The helical strips were approximately 2 cm long and 2-3 mm wide and were suspended in a series of 10 ml isolated organ baths. Tissues were equilibrated under 1 gram tension in a modified Krebs-Henseleit (Krebs') solution at 37°C for at least 90 min before initiating the experiment. The composition of Krebs' solution was as follows (mM): NaCl, 115.21; KCl, 4.70; $CaCl_2$, 1.80; $MgSO_4$, 1.16; KH_2PO_4, 1,18; $NaHCO_3$, 22.14; dextrose, 7.88. Disodium ethylenediamine tetracetic acid (EDTA, 0.03 mM) was added to suppress oxidation of amines. The Krebs' solution was constantly oxygenated with a mixture of oxygen-carbon dioxide (95:5).

Contractions were recorded isotonically. Concentration-response data were obtained by cumulative additions of the agonists in approximately one-half log increments (van Rossum, 1963).

Determination of agonist potencies

Concentration-response data to serotonergic agonists were obtained after each tissue had been exposed to a series of concentrations of KCl and the maximum response to KCl (150mM) was set as the 100% response. EC_{50} values for agonists in the experiment were recorded at the molar concentration where the curves intersected the 50% level of the response axis to that agonist. Relative potencies were calculated by comparing the concentration of agonist required to produce an equivalent contraction as that to 5-HT at the 20% response. No calculations were possible for 2-MPP since its maximum response did not reach the 20% level in this study.

Determination of agonist dissociation constant (K_A or K_P) and relative efficacy (e_r)

K_A value for 5-HT was determined as described by Furchgott and Bursztyn (1967). Dibenamine was used to inactivate a fraction of the receptors. After fractional inactivation of the receptors by dibenamine (7.5×10^{-8}M, for 15 min), the bath fluid was changed 5 or 6 times over 30 minutes. The reciprocals of the concentration of 5-HT before dibenamine treatment (1/[A]) were plotted against the reciprocals of the corresponding equieffective concentrations after the treatment (1/[A']). From the slope and intercept of the straight line fitting the points, the value for K_A and for the fraction of active receptors remaining (q) was calculated on the basis of the equation (Furchgott, 1966):

$$\frac{1}{[A]} = \frac{1-q}{qK_A} + \frac{1}{q[A']} \qquad (1)$$

according to which K_A equals (slope - 1)/intercept, and q equals 1/slope.

DOM, 2-methyl-5-HT, 8-OH-DPAT and the phenylpiperazines (TFMPP and mCPP) were partial agonists on the umbilical vein. The technique of Waud (1969) was initially used to determine the dissociation constant (K_p) for partial agonists. However, it was not applicable in the present study since the umbilical vein was found not to have a large spare receptor capacity for the full agonist, 5-HT [see Kenakin (1984) for a discussion of this requirement]. Therefore, the procedure developed by Gero and Tallarida (1977) was used to determine the K_p of partial agonists by using the following equation:

$$K_p = \frac{K_A \cdot (Ap - Ai) \cdot Pi}{(Ap + K_A) \cdot Ai} \qquad (2)$$

where K_A was the dissociation constant obtained for 5-HT (see above), Ap was the concentration of the full agonist A (i.e., 5-HT) producing a response equal to the maximum of the respective partial agonist p and Ai and Pi were any other pair of concentrations giving a matching contractile response.

The relative affinities of the agonists for serotonergic receptors as compared to 5-HT were obtained by dividing the dissociation constant (K_A) obtained for 5-HT by those Kp constants obtained for the other agonists.

The dissociation constants (K_A or Kp) determined were then used to

calculate the respective fractional occupation of receptors by each agonist for each concentration used in establishing the control concentration-response curves. The fractional occupancy was calculated from the equation (Furchgott and Bursztyn, 1967):

$$\frac{[RA]}{[R_T]} = \frac{[A]}{[A] + k_A} \qquad (3)$$

where [RA] is the concentration of the receptor-agonist complex, $[R_T]$ is the total concentration of receptors, and K_A (or Kp) is the agonist dissociation constant.

The contractile response data for each agonist were then replotted to show response as a function of log $[RA]/[R_T]$. The antilog of the distance between two curves along the abscissa was taken as the ratio of the intrinsic efficacy of the second agonist to that of 5-HT (Furchgott and Bursztyn, 1967), and thus was a measure of e_r, the relative efficacy of the second agonist as compared to 5-HT.

In all experiments, cocaine (3×10^{-6}M) was added to block uptake mechanisms (Dyer, 1970), phentolamine (10^{-7}M) to inhibit α-adrenergic receptors and iproniazid (0.36 mM) to block monoamine oxidase (MAO). Iproniazid was added to the bath for 40 min. and then the tissues were washed 4 times over 30 min with fresh Krebs' solution. Cocaine and phentolamine were added 15 min prior to adding 5-HT.

Determination of the apparent dissociation constant (K_B) of antagonist

Methiothepin, ketanserin, mianserin and MDL 72222 were used in a series of experiments to determine the subtypes of 5-HT receptors involved in contractions of the ovine umbilical vein. Initially a

concentration response relationship was obtained to an agonist. Then antagonists were equilibrated for 1 hour with the tissue before repeating the concentration response relationship to that agonist. The concentration ratio (CR) of the agonist (EC_{50} in the presence of antagonists/EC_{50} in the absence of antagonists) was determined. The time related shift of the agonist response curve was measured in a matched preparation not treated with antagonist. The concentration ratio (CR_T) (EC_{50} at time t/EC_{50} at time 0) was determined from the two control agonist concentration-response curves. The concentration ratio (CR) of the agonist obtained from the antagonist treated tissue was then adjusted according to the following formula: adjusted CR = CR/CR_T. K_B was calculated from the relationship (Furchgott, 1972):

$$K_B = \frac{[B]}{CR - 1} \qquad (4)$$

where [B] is the molar concentration of the antagonist and CR is the adjusted concentration ratio for the agonist as described above. Cocaine, phentolamine and iproniazid were also used as described above.

Evaluation of α-adrenergic receptor stimulation

Experiments were performed to evaluate the possibility that α-adrenergic receptor stimulation was involved in the response to 5-HT. Pairs of adjacent strips from a single umbilical vein were studied to compare the antagonism of prazosin and yohimbine to 5-HT and norepinephrine (NE). The strips were first exposed to KCl (150mM) and then washed to permit relaxation to the original resting level. Prazosin or yohimbine were added to the bath and allowed to equilibrate

with the tissue for 20 minutes. The preparations were then challenged with a series of cumulative additions of 5-HT or NE in the presence of the antagonists. The data were plotted to determine the displacement of the log concentration-response curve for the agonists in the presence of the antagonists.

Drugs

The following drugs were used: cocaine HCl, serotonin creatinine sulfate (Sigma Chemical Co., St. Louis, MO); R(-)-2,5-dimethoxy-4-methyl-amphetamine (National Institute of Drug Abuse, Rockville, MD); 8-hydroxy-dipropylaminotetralin (8-OH-DPAT), α-methyl-5-hydroxytryptamine (α-methyl-5-HT), 2-methyl-5-hydroxytryptamine (2-methyl-5-HT), m-trifluoromethyl-phenylpiperazine HCl (TFMPP), 1-(3-chlorophenyl) piperazine HCl (mCPP), 1-(2-methoxyphenyl) piperazine HCl (2-MPP), 3-tropanyl-3,5-dichlorobenzoate (MDL 72222), mianserin HCl (Res. Biochem. Inc., Natick, Massachusetts). Ketanserin tartrate (Jansson, Beerse, Belgium); iproniazid phosphate, methiothepin maleate (Hoffmann-LaRoche, Nutley, NJ); prazosin HCl (Pfizer Inc., Brooklyn, NY); yohimbine HCl (Merck, Rahway, NJ); phentolamine methane sulfate (CIBA Pharmaceutical Company, Summit, NJ); dibenamine HCl (Smith, Kline and French Lab., Philadelphia, PA); l-norepinephrine bitartrate (Calbiochem Behring Corp., La Jolla, CA). Drugs were dissolved in saline, except for dibenamine and MDL 72222, which were dissolved in alcohol and diluted in saline just prior to use.

Data were expressed as means ± S.E.; for each experiment, n refers to the number of sheep from which vessels were taken. The student's t-test was used for statistical analysis of the difference of means.

Results

Contractions of ovine umbilical vein to serotonergic agonists

Contractions produced by serotonergic agonists were compared to those by potassium chloride (150 mM). The concentration-response curves to serotonergic agonists are illustrated in Fig. 1. The threshold concentration of DOM was lower than that of 5-HT. Other agonists required a higher concentration to produce a threshold response. In Table 1, the EC_{50} values and potency ratios were presented. The potency order of the agonists was determined to be DOM > 5-HT > α-methyl-5-HT > mCPP > TFMPP > 8-OH-DPAT = 2-methyl-5-HT. DOM was 3.4 times more potent than 5-HT but only produced 84% of the maximum response to that of 5-HT. The maximum contractile response elicited by the other agonists was significantly less than that obtained to 5-HT. 2-MPP caused only a minimal response, about 5 percent of that to 5-HT.

Agonist dissociation constant (K_A or K_P)

The response to 5-HT before and after exposing the tissues to dibenamine (7.5×10^{-8}M for 15 min) is presented in Fig. 2. Dibenamine reduced the maximal response to 5-HT about 40%. The inset (Fig. 2) illustrates a double reciprocal plot of equieffective concentrations of 5-HT before (1/[A]) and after (1/[A']) dibenamine treatment. The mean K_A for the individual tissues was $4.25 \pm 1.17 \times 10^{-7}$M (n=7). The fraction of receptors still active after dibenamine (i.e., the q value) was determined as described in 'Methods'. The mean q value for 5-HT was 0.26 ± 0.09 (n=7).

The dissociation constants (Kp) for the partial agonists DOM, 8-

Fig. 1. Concentration response relationship for serotonergic agonists. Results are illustrated as the $\bar{x} \pm$ SE. of the tissues from 3 to 7 animals and are expressed as a percentage of the contraction obtained to 150mM KCL

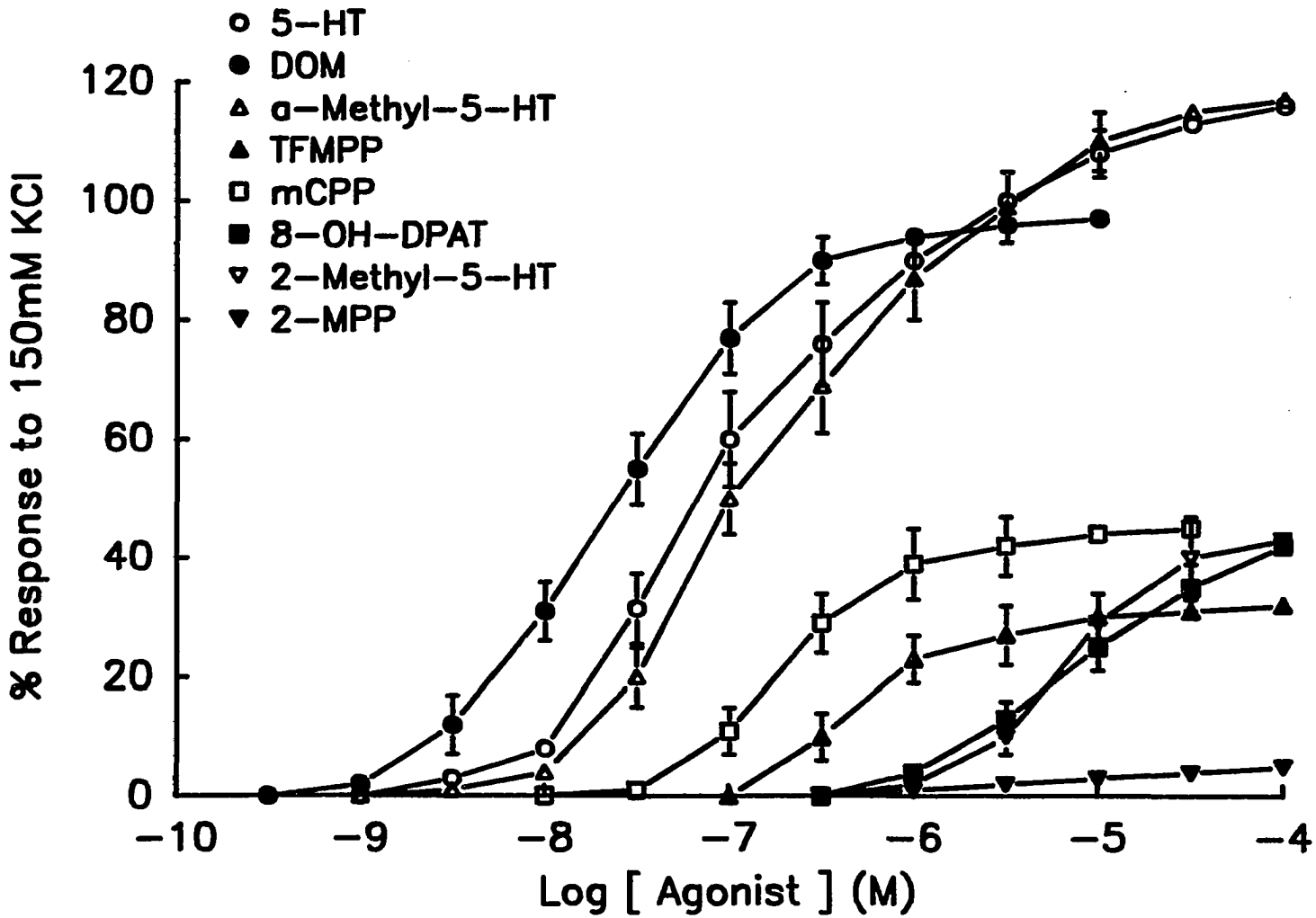

5–HT
DOM
α–Methyl–5–HT
TFMPP
mCPP
8–OH–DPAT
2–Methyl–5–HT
2–MPP
% Response to 150mM KCl
120
100
80
60
40
20
0
–10
–9
–8
–7
–6
–5
–4
Log [Agonist] (M)

Table 1. Comparative properties of serotonergic agonists on the ovine umbilical vein

Agonist	N[a]	EC_{50}[b]	Relative Potency[c]	Approximate EC_{100}[d]	Maximum Response as a Percentage of the Response to 150mM KCl
		M (x 10^{-7})		M	%
5-HT	7	0.95 ± 0.09	1.000	1.0×10^{-4}	116
DOM	7	0.22 ± 0.03	3.400[e]	1.0×10^{-5}	97
α-Methyl-5-HT	6	1.65 ± 0.11	0.548[e]	1.0×10^{-4}	117
mCPP	4	2.23 ± 0.43	0.096[e]	3.0×10^{-5}	45
TFMPP	4	5.62 ± 1.42	0.021[e]	1.0×10^{-4}	32
8-OH-DPAT	4	63.09 ± 15.14	0.003[e]	1.0×10^{-4}	42
2-Methyl-5-HT	3	67.81 ± 18.90	0.003[e]	1.0×10^{-4}	42
2-MPP	3	121.13 ± 40.14		1.0×10^{-4}	5

[a]N is the number of animals.

[b]EC_{50} is the effective concentration to produce 50% of the maximal response to the respective agonist.

[c]Relative potency was calculated at the EC_{20} (see Methods). 5-HT is arbitrarily set at 1.

[d]EC_{100} is the effective concentration to produce the maximal response to the respective agonist.

[e]Significantly different from 1 ($P<0.05$).

Fig. 2. Effect of treatment with dibenamine on 5-HT-elicited contractions of ovine umbilical vein. Contractions were obtained before (open circles) and after (closed circles) exposure to 7.5 x 10^{-8}M dibenamine for 15 min. Dibenamine was washed out of the tissues before obtaining the second concentration-responsive relationship to 5-HT. Each point represents the $\bar{x} \pm$ SE. of tissues from 7 animals. Inset: plot of the reciprocals of equieffective concentrations of 5-HT before (1/[A]) and after (1/[A']) dibenamine treatment

% Maximal Contraction
0
20
40
60
80
100
Log [5-HT] (M)
-3
-4
-5
-6
-7
-8
-9
1/[A'] X 10^5
0
40
80
120
160
1/[A] X 10^6
0
20
40
60
80

OH-DPAT, 2-methyl-5-HT and the phenylpiperazines (TFMPP and mCPP) are presented in Table 2. The relative affinity of partial agonists were calculated by dividing the estimated K_A of 5-HT by the Kp of the partial agonist (Table 2). Of the five agonists studied, DOM and mCPP had an affinity for the serotonergic receptor site which was greater than that of 5-HT (Table 2). The affinities of the other three agonists were lower than that of 5-HT (Table 2).

Agonist relative efficacy (e_r)

Fig. 3 presents the results of replotting agonist relative response against log $[RA]/[R_T]$. The fraction of receptors occupied ($[RA]/[R_T]$) for each agonist at each concentration employed in obtaining the complete concentration-response curves was calculated as described in Methods. The curve for 5-HT indicated that 50% of the maximum response was obtained when about 17% of the receptors were occupied by 5-HT. However, about 90% of the receptors had to be occupied to obtain 90% of the maximum response. The relative efficacies of DOM, 2-methyl-5-HT, 8-OH-DPAT, mCPP and TFMPP to 5-HT were obtained as the antilog of the distance between the respective agonist and 5-HT along the log $[RA]/[R_T]$ axis. The mean values are presented in Table 2.

Correlation between agonist sensitivity and potency and agonist affinity and efficacy

There is an excellent correlation between the sensitivity (pD_2 = - log EC_{50}) and the affinity (pK_A = - log K_A) of the serotonergic agonists in the ovine umbilical vein (pD_2 vs. pK_A) (Fig. 4). The correlation coefficient is 0.966 ($P < 0.001$). The slope of the regression line relating the sensitivity to affinity is 0.84, which is not significantly

Table 2. Comparison of dissociation constant (K_A or K_p), relative efficacy (e_r) and relative affinity for the agonists acting on the serotonergic receptors in the ovine fetal umbilical vein

Agonist	N[a]	Dissociation Constant	Relative Efficacy[b]	Relative Affinity[c]
		M (x 10^{-7})		
5-HT	7	4.25 ± 1.17	1.00	1.00
DOM	7	0.85 ± 0.24	0.63[d]	5.00[d]
mCPP	4	2.64 ± 0.73	0.11[d]	1.61
TFMPP	4	5.40 ± 1.62	0.07[d]	0.79
8-OH-DPAT	4	113.89 ± 29.74	0.12[d]	0.04[d]
2-Methyl-5-HT	3	89.44 ± 21.80	0.11[d]	0.05[d]

[a]N is the number of animals.
[b,c]5-HT is arbitrarily set at 1.
[d]Significantly different from 1 ($P<0.05$).

Fig. 3. Contractions to serotonergic agonists plotted as a function of log $[RA]/[R_T]$ in the ovine umbilical vein. Receptor occupancy $[RA]/[R_T]$ at a given concentration of agonist was calculated as described under "Methods" using the mean dissociation constant obtained for each agonist, as given in Table 2. Contractile responses were taken from the results illustrated in Figure 1

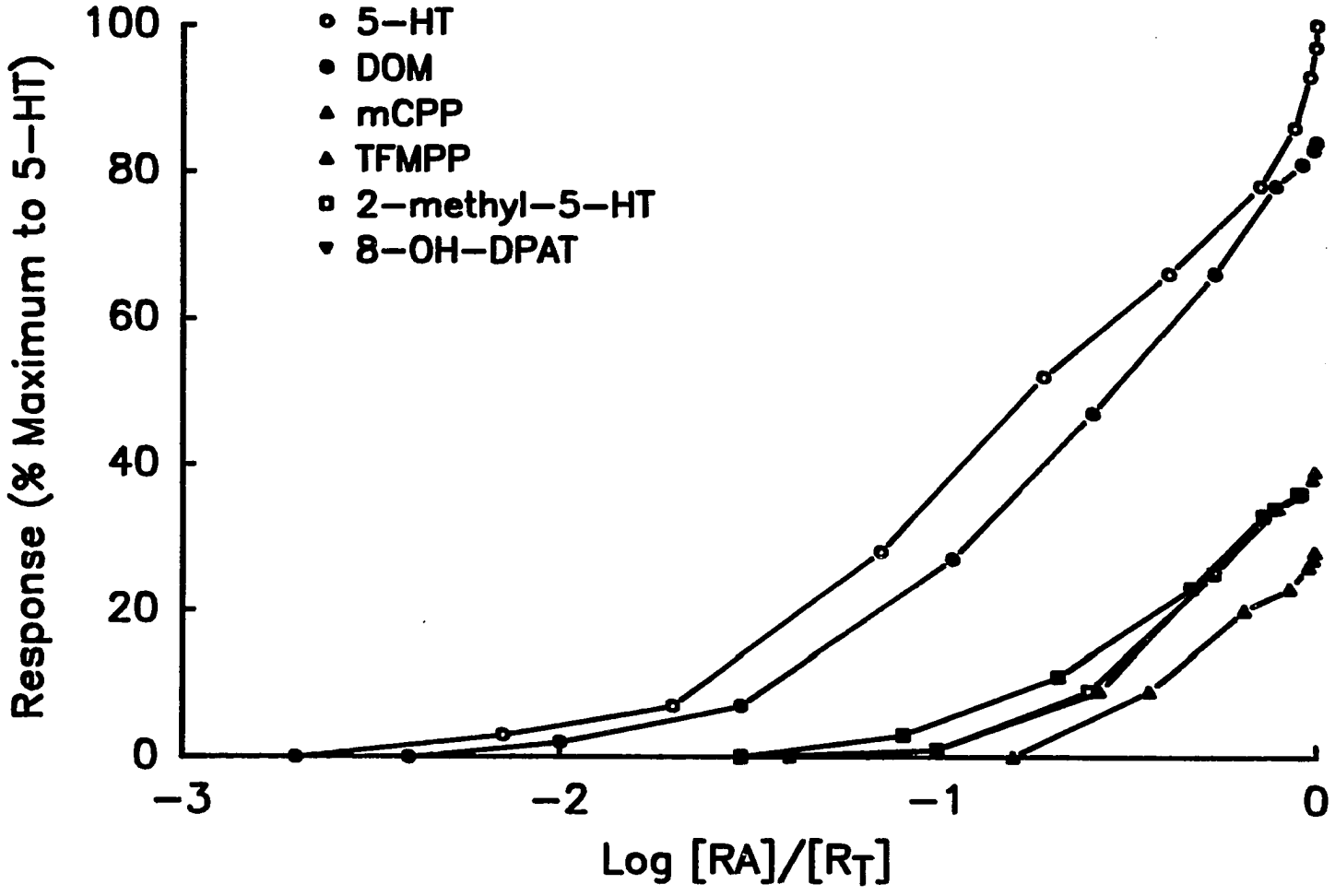
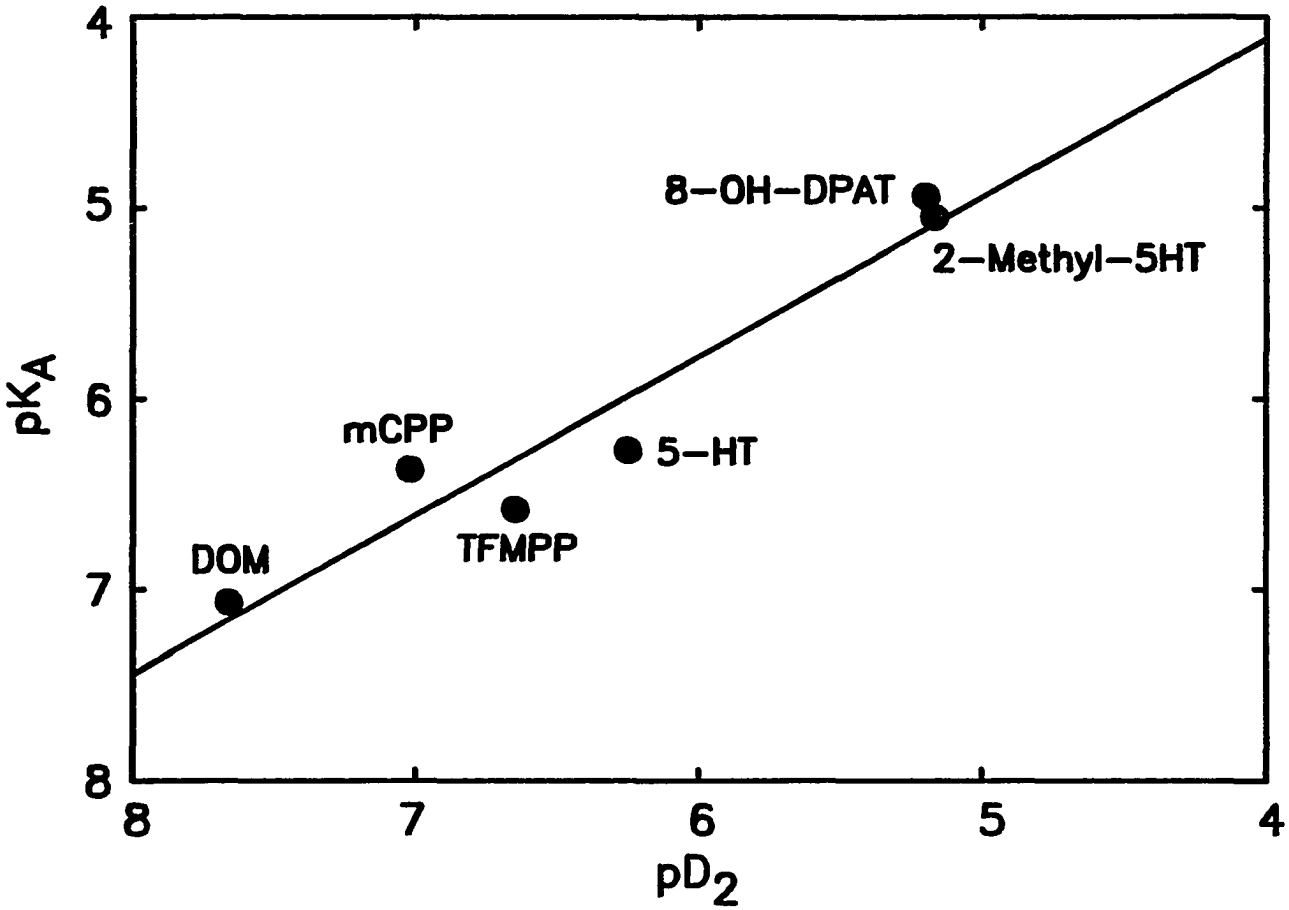

100
80
60
40
20
0
−3
−2
−1
0
Response (% Maximum to 5−HT)
Log [RA]/[RT]
5−HT
DOM
mCPP
TFMPP
2−methyl−5−HT
8−OH−DPAT

Fig. 4. Correlation between pK_2 (- log EC_{50}) and pK_A (- log K_A) of serotonergic agonists in the ovine umbilical vein. Correlation coefficient $r = 0.966$ ($P < 0.001$). Slope = 0.84 which is not significantly different from unity


4
5
6
7
8
pK_A
8-OH-DPAT
2-Methyl-5HT
5-HT
mCPP
TFMPP
DOM
8
7
6
5
4
pD_2

different from unity. Fig. 5 (left panel) shows that there is a good correlation between the relative potency (RP) and relative affinity (RA) of the serotonergic agonists where $r = 0.896$ ($P < 0.01$). The slope of the regression line relating RP to RA is 0.6, which is significantly different from unity. The correlation between RP and relative efficacy (RE) of the agonists is also significant with $r = 0.824$ ($P < 0.05$) (Fig. 5, right panel). The slope of the regression line is 0.32, which is significantly different from unity.

Competitive antagonists

Four antagonists of 5-HT receptors were used in this investigation. These included: methiothepin, ketanserin, mianserin and MDL 72222. Figure 6 presents the results of the inhibitory effects of these antagonists on the 5-HT-induced contractions on the ovine umbilical vein. Apparent dissociation constants, K_B values, are presented in Table 3. The potencies for inhibition of 5-HT-induced contractions were very similar among these antagonists except for MDL 72222, which was much less potent.

Contractile responses to DOM, mCPP, 8-OH-DPAT and 2-methyl-5-HT were effectively blocked by ketanserin (10^{-8}M). The dissociation constants (K_B) of ketanserin against these agonists were determined to be: DOM, 2.78 ± 0.85 nM (n=3); mCPP, 1.45 ± 0.51 nM (n=3); 8-OH-DPAT, 3.47 ± 1.12 nM (n=3); 2-methyl-5-HT, 1.99 ± 0.74 nM (n=3).

Effect of blockade of α-adrenergic receptors

The ability of 5-HT to stimulate α-adrenergic receptors was evaluated in umbilical vein strips pretreated with prazosin or yohimbine. Paired tissues from the same vein were used to compare the

Fig. 5. Correlation between the relative potency (RP) and relative affinity (RA) (left panel) and relative efficacy (RE) (right panel) of the serotonergic agonist in the ovine umbilical vein. Correlation coefficients for relating RP to RA and RE are 0.896 ($P < 0.01$) and 0.824 ($P < 0.05$), respectively. Slopes for the regression lines relating RP to RA and RE are 0.60 and 0.32, respectively

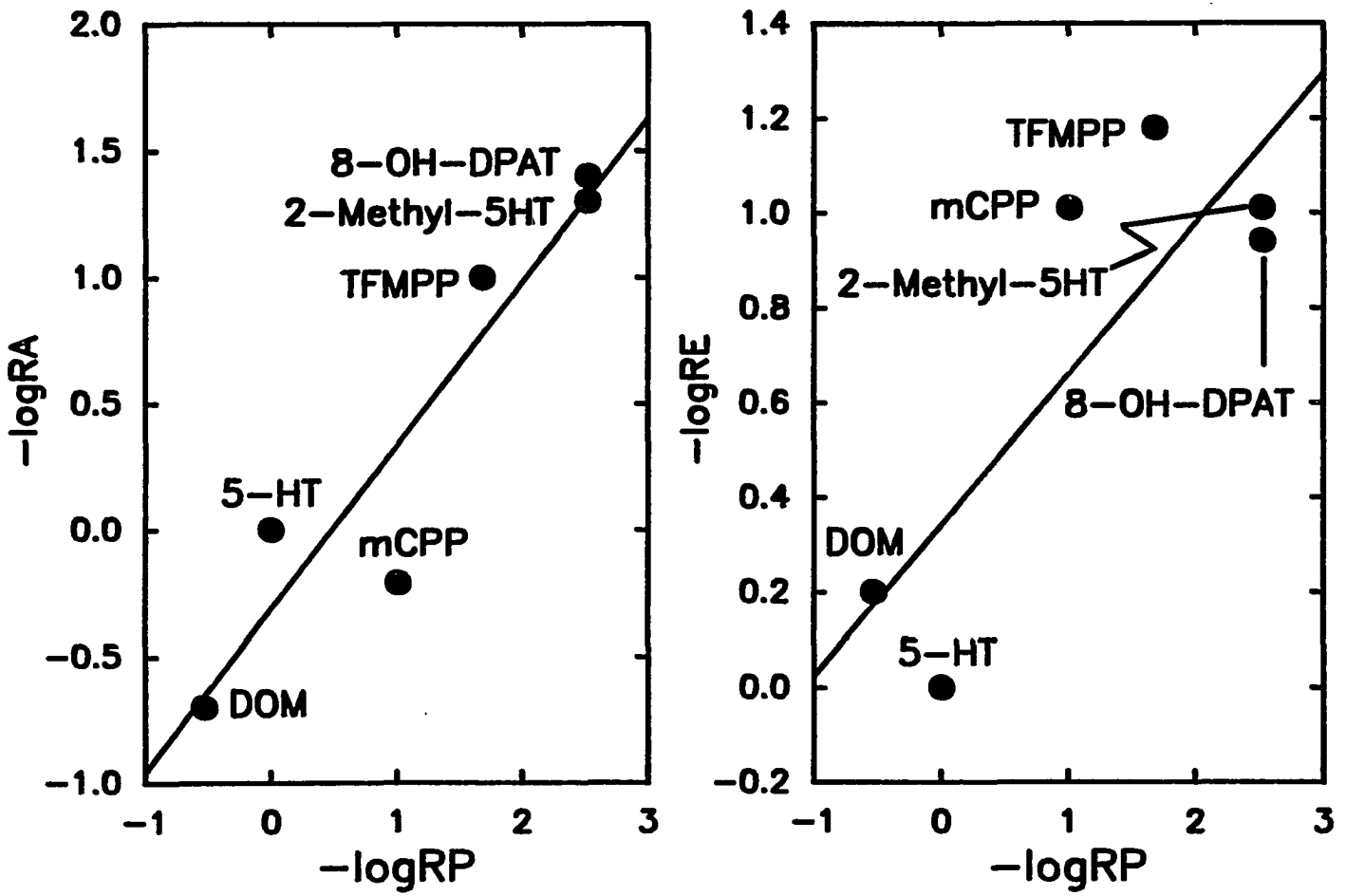

2.0
1.5
1.0
0.5
0.0
−0.5
−1.0
−logRA
8−OH−DPAT
2−Methyl−5HT
TFMPP
5−HT
mCPP
DOM
−1
0
1
2
3
−logRP
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
−0.2
−logRE
TFMPP
mCPP
2−Methyl−5HT
8−OH−DPAT
DOM
5−HT
−1
0
1
2
3
−logRP

Fig. 6. Cumulative concentration-response curves for 5-HT obtained with ovine umbilical vein in the absence and presence of various serotonergic antagonists. Each point represents the $\bar{x} \pm SE$. of the tissues from 3 to 4 animals

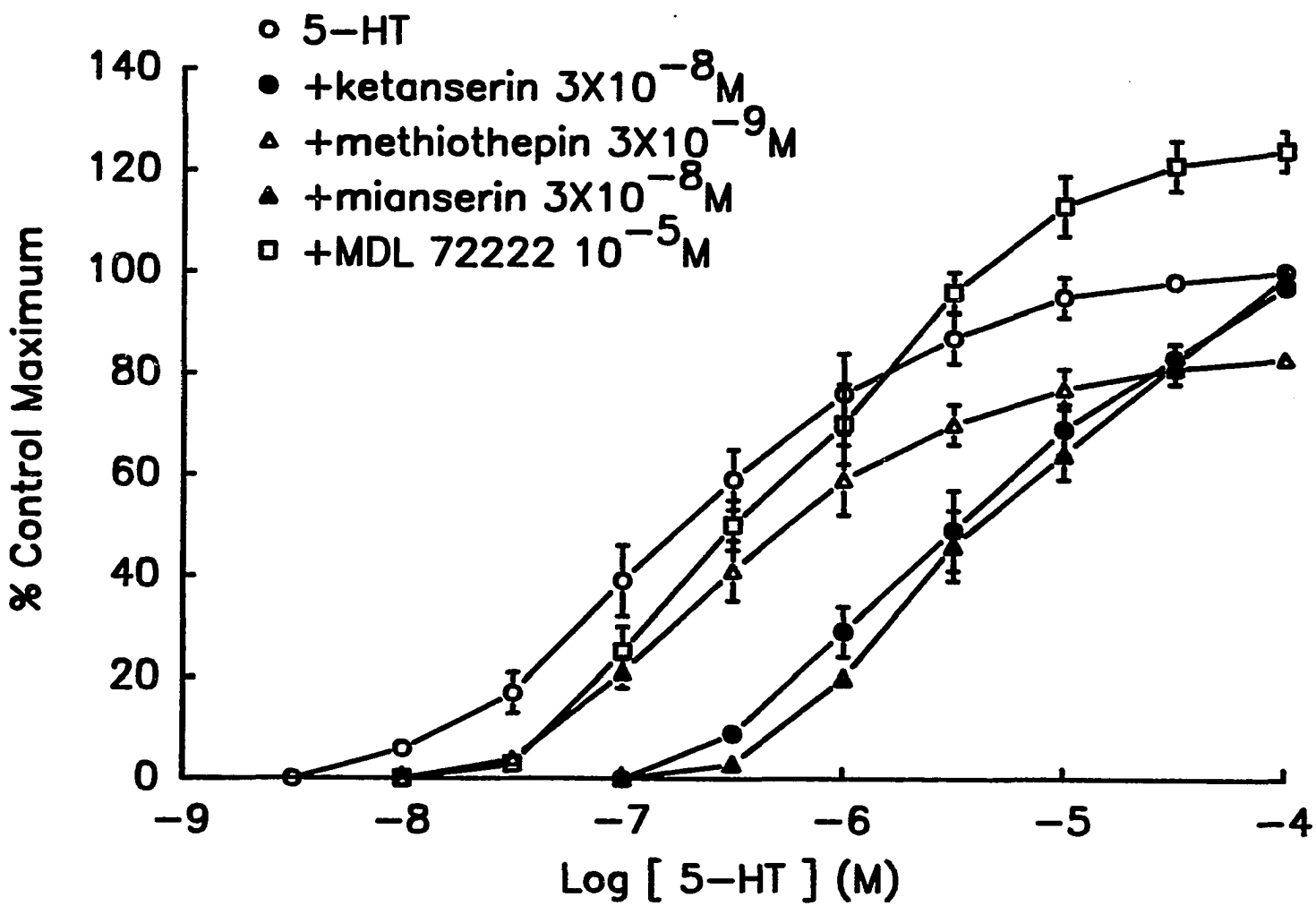

5–HT
+ketanserin 3X10^{-8}M
+methiothepin 3X10^{-9}M
+mianserin 3X10^{-8}M
+MDL 72222 10^{-5}M
% Control Maximum
140
120
100
80
60
40
20
0
−9
−8
−7
−6
−5
−4
Log [5–HT] (M)

Table 3. Effect of antagonists on responses to 5-HT on the isolated ovine umbilical vein

Antagonist	Conc.	N[a]	Emax[b]	K_B[c]
	M		%	nM
Ketanserin	3×10^{-8}	4	98 ± 2.13	2.17 ± 0.36
Methiothepin	3×10^{-9}	4	83 ± 3.47	1.98 ± 0.48
Mianserin	3×10^{-8}	4	99 ± 3.26	1.37 ± 0.55
MDL 72222	1×10^{-5}	3	124 ± 4.4	13833 ± 5674

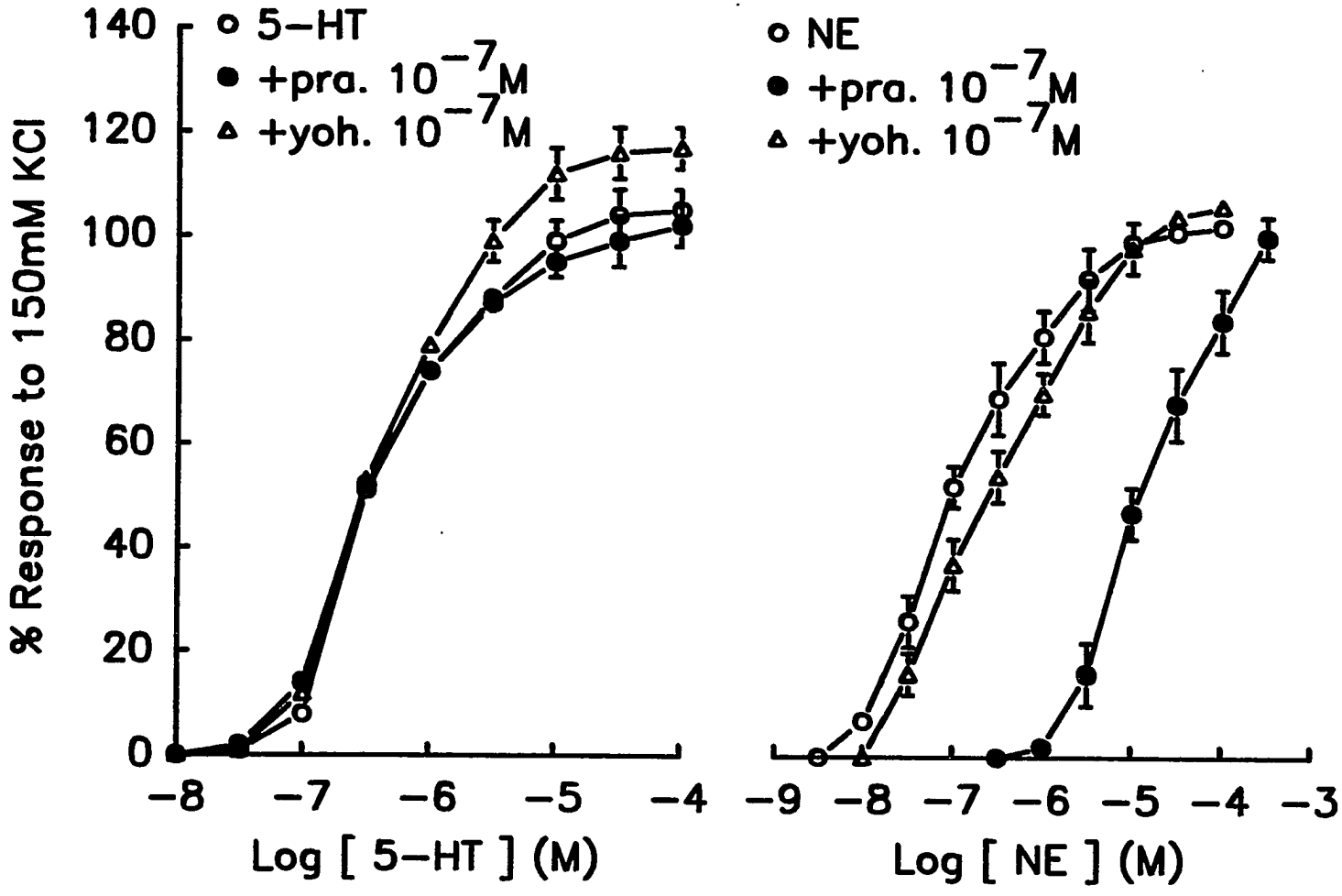
[a]N is the number of animals.
[b]Maximum contraction to 5-HT in the presence of antagonist as a percentage of the maximum obtained before exposing tissues to the antagonist.
[c]Calculated for each tissue at each concentration of antagonist according to the equation K_B = [antagonist]/concentration ratio-1.

antagonism of prazosin and yohimbine to NE or 5-HT. Fig. 7 illustrates the results of this experiment. Neither prazosin (10^{-7}M) nor yohimbine (10^{-7}M) antagonized responses to 5-HT. In contrast, NE-induced contractions were effectively antagonized by the same concentration of yohimbine and prazosin. Yohimbine (10^{-7}M) increased the EC_{50} of NE 2-fold, while prazosin (10^{-7}M) increased it 112-fold.

Discussion

Norepinephrine produced a concentration-dependent contraction of the isolated ovine umbilical vein which was antagonized effectively by prazosin and less so by yohimbine. This result indicates that α-adrenergic receptors are present in this tissue. It has been demonstrated that in the cat spleen (Innes, 1962) and rabbit central ear artery (Apperley et al., 1976; Purdy et al., 1980) that α-adrenergic receptors can mediate a 5-HT response. In the present study, prazosin, with a reported K_B of 3.51nM for α-receptors in the rabbit aorta (Furchgott, 1980), was not effective at 100nM in antagonizing the 5-HT response. Similarly, yohimbine (10^{-7}M) was without inhibitory effects on the 5-HT-induced contractions. We conclude that contractions to 5-HT in the ovine umbilical vein do not involve activation of α-adrenergic receptors. This conclusion is in accord with that found in the ovine uterine artery (Zhang and Dyer, 1989a) and in the rabbit aorta (Stollak and Furchgott (1984). That α-adrenergic receptors can mediate a 5-HT response in some blood vessels but not in others indicates that there could be differences in the properties and/or relative numbers of α-

Fig. 7. Cumulative concentration-response curves to 5-HT (left panel) and norepinephrine (right panel) on the ovine umbilical vein in the absence and presence of prazosin (pra) and/or yohimbine (yoh). Each point represents the $\bar{x} \pm$ SE. of the tissues from 4 animals


% Response to 150mM KCl
140
120
100
80
60
40
20
0
o 5-HT
● +pra. 10⁻⁷M
▲ +yoh. 10⁻⁷M
-8
-7
-6
-5
-4
Log [5-HT] (M)
o NE
● +pra. 10⁻⁷M
▲ +yoh. 10⁻⁷M
-9
-8
-7
-6
-5
-4
-3
Log [NE] (M)

receptors from one tissue to another. It is possible that 5-HT could be a very weak partial agonist at α-receptors but in tissues like the ovine umbilical vein there may not be a large enough α-receptor reserve to permit generation of a response with a low-efficacy partial agonist. In fact, we have demonstrated that there is no receptor reserve for α-receptors in the ovine umbilical artery since to obtain a 50% response to NE required about 50% of the α-receptors to be occupied (Zhang and Dyer, unpublished data).

The K_A value for 5-HT determined in this study was not significantly different from our previous finding in ovine uterine artery and umbilical artery (Zhang and Dyer, 1989a, 1989b) and is comparable to its binding affinity at 5-HT_2 binding sites in brain tissue (k_i = 400-1000nM) (Glennon, 1987). Based on the determined K_A of 425nM, 5-HT would occupy 17% of the total functional receptor pool to produce one-half of the maximum response. However, about 90% of the receptors would need to be occupied by 5-HT when 90% of the maximum response is produced, suggesting a lack of any appreciable effective receptor reserve. The lack of an effective receptor reserve in the ovine umbilical vein agrees with our recent finding in the ovine uterine and umbilical artery (Zhang and Dyer, 1989a, 1989b). Evidence suggesting a lack of spare receptors for 5-HT_2 receptors has been obtained by Cohen et al. (1986) in the rat jugular vein and uterus.

Differences in the agonist sensitivity in producing the biological response of a tissue may result from the variation in the agonist affinity for the receptor as well as the number of the receptors involved and the vents that follow receptor occupation (see discussion

by Bevan et al., 1989). It has been shown that the sensitivity of rabbit arteries to NE was totally dependent on the affinity of NE for α-adrenergic receptors, while in rat arteries only 60% of NE sensitivity was due to the variation in the receptor affinity and with 40% coming from the variation in the receptor density (Oriowo et al., 1989). In the present study, an excellent correlation between the sensitivity and the affinity of serotonergic agonists (pD_2 vs. pK_A) was observed. The slope of the regression line relating the sensitivity to the affinity is not significantly different from unity. This suggests that the variation in the sensitivity of the serotonergic agonists in the ovine umbilical vein is primarily due to the variation in their affinities for 5-HT_2 receptors. As discussed by Kenakin (1984), variation in the relative potencies among agonists may result from the difference in their affinities and/or their relative efficacies. This is supported by the observation in this study that there are good correlations between the relative potency of the serotonergic agonists and their relative affinity and efficacy. The slopes of the regression lines relating the relative potency to the relative affinity and the relative efficacy are 0.6 and 0.32, respectively. This suggests that about a 60% variation in the serotonergic agonist potency is due to the variation in their relative affinity for 5-HT_2 receptors and about 30% the variation in the relative potency comes from the variation in their relative efficacy.

In comparison to 5-HT, α-methyl-5-HT behaved as a full agonist and all others as partial agonists. The relative efficacy of DOM, mCPP, TFMPP, 8-OH-DPAT and 2-methyl-5-HT was lower than that of 5-HT in the umbilical vein. The classification of DOM as a partial agonist in the

present study is in accord with that found in the ovine umbilical artery (Zhang and Dyer, 1989b) and in brain tissue (Sanders-Bush et al., 1988), but differs from that found in the ovine uterine artery (Zhang and Dyer, 1989a). The reason why DOM is a full agonist in some tissues and a partial agonist in others is not clear at this point. Since DOM had a lower efficacy than that of 5-HT in the ovine umbilical vein, its greater potency when compared to 5-HT is probably linked to its greater affinity for 5-HT receptors.

Ketanserin, a selective 5-HT_2 receptor antagonist (Leysen et al., 1981, Van Neuten et al., 1981; Leysen et al., 1982) caused a parallel shift of the concentration-effect curve for 5-HT to the right. The apparent K_B value (2.17 nM) for ketanserin obtained in this study was comparable to our recent finding in the ovine uterine artery (2 nM) and umbilical artery (0.4 nM) (Zhang and Dyer, 1989a, 1989b) and those (0.2 to 7 nM) reported for other blood vessels (Cohen et al., 1983; Cohen, 1986; Humphrey, 1984; Van Nueten et al., 1982; Van Nueten et al., 1981). This indicates that 5-HT evoked contractions of the umbilical vein are via the 5-HT_2 receptor. Mianserin is generally regarded as selective for 5-HT_2 as opposed to 5-HT_1 receptors, and [^{3}H] mianserin has been used as a ligand for 5-HT_2 sites (Peroutka and Snyder, 1981). Cohen (1984) demonstrated that mianserin acted as a typical competitive antagonist of 5-HT_2 receptors in the rat jugular vein. The present finding that mianserin potently antagonized 5-HT-induced contractions in the umbilical vein further supports the contention that 5-HT_2 receptors are involved in 5-HT-induced contractions in this tissue. The contractile response to DOM was effectively blocked by ketanserin. The

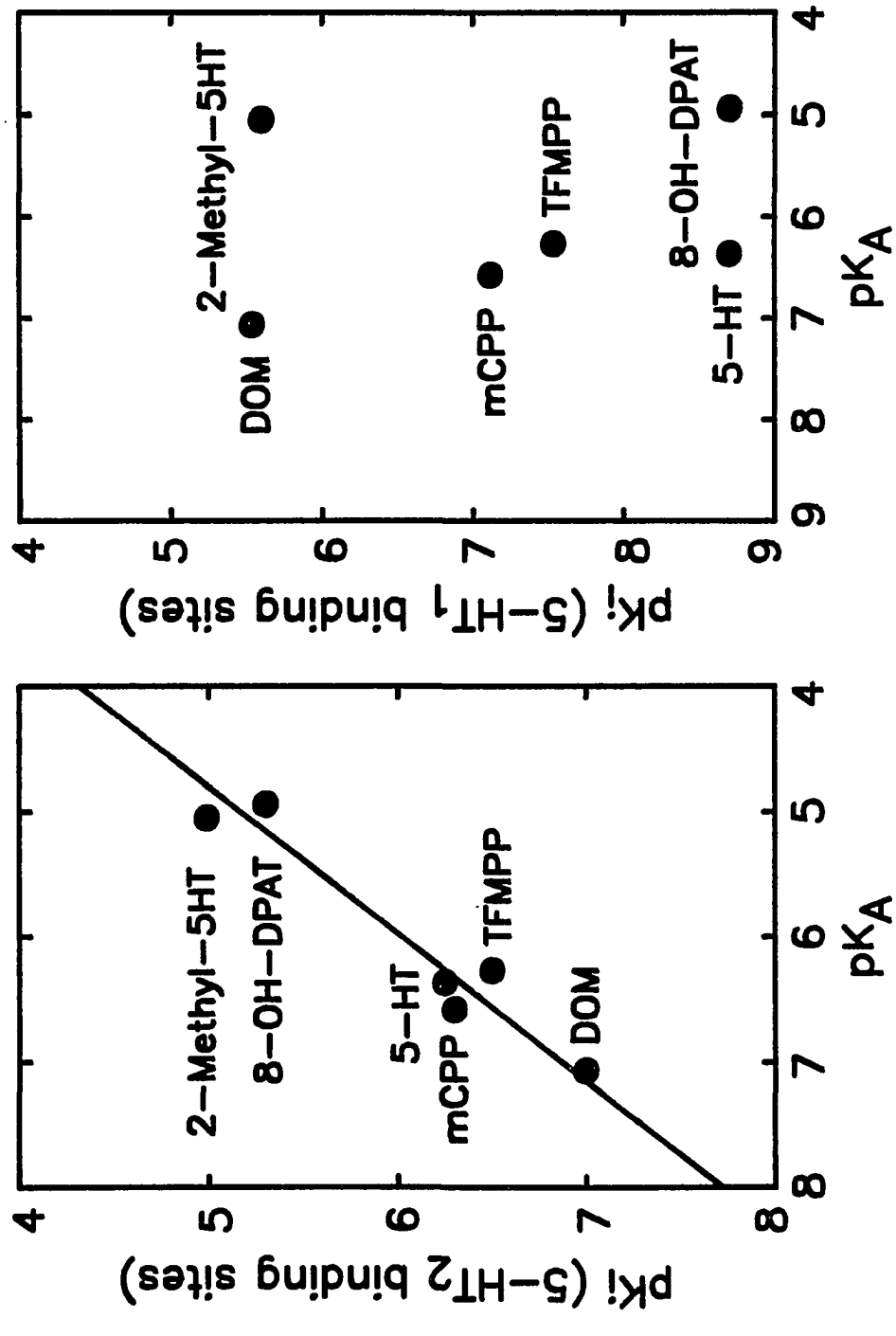
dissociation constant (K_B) of ketanserin vs. DOM (2.78 nM) was not significantly different from that (2.17 nM) of ketanserin vs. 5-HT, indicating that DOM acts on the same 5-HT receptors as 5-HT does, i.e., 5-HT_2 receptors. That both 5-HT and DOM activate 5-HT_2 receptor is in accord with our findings in the ovine uterine and umbilical artery (Zhang and Dyer, 1989a, 1989b) and those by Glennon and co-workers (1983, 1984) in brain tissues.

There is an excellent correlation ($r = 0.964$, $P < 0.001$) between the pK_A of the serotonergic agonists in this study and their affinities (pK_i) at 5-HT_2 binding sites in the CNS reported by other authors (Fig. 8, left panel). In contract, there is a negative correlation ($r = -0.205$) between the pK_A of the agonists and their pK_i at 5-HT_1 binding sites in the CNS (Fig. 8, right panel). These results suggest that the 5-HT receptors mediating the contractions produced by the agonists in this study are the same type of 5-HT_2 binding sites in the CNS.

It is unlikely that contractions produced by 8-OH-DPAT in the present study are mediated by 5-HT_{1A} receptors, since ketanserin effectively blocked contraction produced by 8-OH-DPAT. The dissociation constant (K_B) of ketanserin against 8-OH-DPAT (3.47 nM) was not significantly different from that of ketanserin vs. 5-HT (2.17 nM), suggesting that contractions produced by 8-OH-DPAT in the ovine umbilical vein were mediated by 5-HT_2 receptors. This is consistent with our finding in the ovine uterine artery (Zhang and Dyer, submitted for publication).

In the present study, 2-MPP lacked agonistic activity up to 10^{-4}M. The other two phenylpiperazines, mCPP and TFMPP acted as partial

Fig. 8. Correlation between the pK_A of the serotonergic agonists in this study and their pK_i at 5-HT_2 binding sites in brain tissue (labelled by [^{3}H] ketanserin, 5-HT and DOM, Glennon, 1987; TFMPP and 8-OH-DPAT, Taylor et al., 1986; mCPP, Martin and Sanders-Bush, 1982; 2-methyl-5-HT, Engel et al., 1986) or at 5-HT_1 binding sites in brain tissue (5-HT and DOM, labelled by [^{3}H] 5-HT, Glennon, 1987; TFMPP and mCPP, labelled by [^{125}I] ICYP, Glennon, 1987; 8-OH-DPAT, labelled by [^{3}H] 8-OH-DPAT, Glennon, 1987; 2-methyl-5-HT, labelled by [^{3}H] 8-OH-DPAT, Engel, et al., 1986). The correlation coefficient for relating the pK_A to the pK_i at 5-HT_2 binding sites and the pK_i at 5-HT_1 binding sites are 0.964 ($P < 0.001$) and -0.205, respectively


pKi (5-HT2 binding sites)
pKA
2-Methyl-5HT
8-OH-DPAT
5-HT
mCPP
TFMPP
DOM
pKi (5-HT1 binding sites)
pKA
2-Methyl-5HT
DOM
mCPP
TFMPP
5-HT
8-OH-DPAT

agonists at 5-HT receptors in the ovine umbilical vein. The dissociation constants (Kp) of mCPP (0.22 μM) and TFMPP (0.56 μM) in the present study were not significantly different from those of mCPP (0.32 μM) and TFMPP (0.43 μM) found in the ovine umbilical artery (Zhang and Dyer, manuscript in preparation). This suggests that they act on the same 5-HT receptors in the two vessels. We have shown that mCPP and TFMPP acts on 5-HT_2 receptors in the ovine umbilical artery (Zhang and Dyer, manuscript in preparation). In this study, ketanserin effectively blocked contractile responses to mCPP. The dissociation constant (K_B) of ketanserin against mCPP (1.45 nM) was not significantly different from that of ketanserin vs. 5-HT (2.17 nM) in the present study, suggesting that contraction produced by mCPP were mediated by 5-HT_2 receptors in the ovine umbilical vein. The very weak agonist activities of mCPP and TFMPP were also found in the ovine uterine artery (Zhang and Dyer, submitted for publication) and the rat jugular vein and aorta (Cohen and Fuller, 1983), in which they antagonized the contractile responses to 5-HT at 5-HT_2 receptors. The dissociation constants (Kp) of mCPP (0.22 μM) and TFMPP (0.56 μM) as partial agonists in the present study are comparable to the dissociation constants (K_B) of mCPP and TFMPP as antagonists against 5-HT in the ovine uterine artery (0.13 μM and 0.22 μM, respectively) (Zhang and Dyer, submitted for publication) and in the rat jugular vein and aorta (0.04 μM and 0.06 μM, respectively) (Cohen and Fuller, 1983). The much lower agonist activity of these phenylpiperazines in the ovine uterine artery and in the rat jugular vein than in the ovine umbilical vessels may be due to differences in their relative efficacies and/or to differences in the

efficiency of stimulus-response coupling in different vessels (see discussion by Kenakin, 1984).

MDL 72222 was demonstrated by Fozard (1984) to be a potent and selective 5-HT_3 receptor antagonist. In the present study, MDL 72222 (10^{-5}M) produced a slight rightward shift in the concentration-response relationship for 5-HT. The dissociation constant (K_B) of MDL 72222 vs. 5-HT in this study was determined to be 13833 nM, which was about 450 to 45000 times higher than that of MDL 72222 acting on 5-HT_3 receptors (31 to 0.31 nM) (Bradley et al. 1986). It is unlikely that 5-HT_3 receptors are present in the ovine umbilical vein. This is in agreement with that found in the ovine uterine and umbilical artery (Zhang and Dyer, 1989a, 1989b). Contractile responses to 2-methyl-5-HT, a selective 5-HT_3 agonist (Bradley et al., 1986), were effectively blocked by ketanserin. The dissociation constant (K_B) of ketanserin vs. 2-methyl-5-HT (1.99 nM) was not significantly different from that of ketanserin vs. 5-HT (2.17 nM), suggesting that contractions produced by 2-methyl-5-HT are mediated by 5-HT_2 receptors in the ovine umbilical vein.

In summary, 5-HT and DOM were potent agonists and produced contraction of the ovine umbilical vein via 5-HT_2 receptors. Activation of α-adrenergic receptors were not involved in the response to 5-HT. DOM was more potent than 5-HT but only produced 84% of the maximum response to that of 5-HT. Variation in the agonist sensitivity and potency is primarily due to the variation in their affinity for 5-HT receptors. There was no 5-HT receptor reserve in the ovine umbilical vein. 8-OH-DPAT, mCPP and 2-methyl-5-HT were partial agonists and produced contractions by acting on 5-HT_2 receptors. 5-HT_3 receptors

were not present in the ovine umbilical vein.

Acknowledgement

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DISCUSSION

The classification of 5-HT receptors in this study is based on the receptor occupation theory where it is assumed that the occupation of a receptor by a drug leads to a stimulus and a subsequent response. In the present study, the order of the relative potency of 5-HT and the other serotonergic agonists in the uterine artery and the umbilical artery and vein are similar except for the three phenylpiperazine derivatives which are more potent in the umbilical vessels than they are in the uterine artery (Table 1). In all three vessels, DOM is more potent than 5-HT, which is due to its greater affinity for the 5-HT_2 receptor. Differences in relative potency among agonists may result from differences in their relative affinity for receptors and/or their relative efficacy. In this study, the variation in the sensitivity and the potency of the serotonergic agonists on the uterine artery and the umbilical vessels is primarily due to the variation in their relative affinity for the 5-HT_2 receptor and less so to the variation in their relative efficacy.

In many isolated tissues, the relationship between receptor occupancy and tissue response is nonlinear (Furchgott, 1966). Receptor reserve may be different among different agonists and/or different tissues. In the present study, no 5-HT receptor reserve was found in the uterine and umbilical vessels even though the half maximum response was reached when only about 20% of the receptors were occupied by 5-HT. It should not be assumed that for drugs that have no receptor reserve the relationship between receptor occupancy and tissue response is

Table 1. Comparative properties of serotonergic agonists in the ovine uterine artery (UA), umbilical artery (UmA) and umbilical vein (UmV)

Agonist	EC_{50}[a]			Relative Potency[b]			Percentage of Response to 150mM KCl at EC_{100}		
	UA	UmA	UmV	UA	UmA	UmV	UA	UmA	UmV
	M ($\times 10^{-7}$)						%		
5-HT	1.27	1.04	0.95	1.000	1.000	1.000	106	161	116
DOM	0.54	0.10	0.22	2.000	5.000	3.400	103	126	97
α-Methyl-5-HT	1.34	1.25	1.65	0.934	0.714	0.548	106	157	117
8-OH-DPAT	21.40	19.95	63.09	0.025	0.015	0.003	68	98	42
2-Methyl-5-HT	38.80	70.79	67.81	0.014	0.004	0.003	69	74	42
mCPP	321.00	1.54	2.23	0.001	0.280	0.096	42	93	45
TFMPP	535.00	1.58	5.62		0.280	0.021	21	96	32
2-MPP	330.00	79.43	121.13		0.002		2	52	5

[a] EC_{50} is the effective concentration to produce 50% of the maximal response to the respective agonist.

[b] Relative potency is calculated at the concentration of agonists which produce the same effect. Potency of 5-HT is arbitrarily set at 1.

linear (Besse and Furchgott, 1976). The efficiency of the stimulus response mechanisms in different tissues could be different. The following two factors can affect the efficiency of the stimulus response mechanism in a tissue: 1) the number of receptors in the tissue, 2) the second messenger system which translates receptor stimulus into a tissue response. The second messenger system often performs as an amplifier in biological praparations (Ariens and Simonis, 1976; Goldberg, 1975). The formation of glucose by β-adrenergic drugs provides a very good example (Goldberg, 1975). On the other hand, a positive correlation between receptor density and agonist response has also been observed (Bevan et al., 1989; Oriowo et al., 1989).

Selective agonism can provide useful information about the presence or absence of a certain receptor in an unclassified tissue. However, one should be very careful if a judgement is based only on the observation of the presence or absence of a response to a selective agonist. If a tissue does not respond to a selective agonist, it could mean either that the receptor is not present or that the stimulus-response mechanism of the tissue produces insufficient amplification of the receptor stimulus to generate a response. The observation with prenalterol, a β_1 agonist, in the canine coronary artery provides a good example that an inadequately efficient stimulus-response mechanism in a tissue could lead to a wrong conclusion about the presence or absence of a certain receptor by using a selective agonist (Baron et al., 1972; Kenakin and Beek, 1980, 1982). In this study, the lack of or weak agonist activity of the phenylpiperazines in the uterine artery could be due to an inadequately efficient stimulus-response mechanism in this

tissue. On the other hand, if a selective agonist produces a response in a tissue, a distinction should be made between selectivity and specificity. In the present study, a 5-HT_3 receptor agonist (2-methyl-5-HT), a 5-HT_{1A} receptor agonist (8-OH-DPAT) and several 5-HT_{1B} receptor agonists (2-MPP, TFMPP and mCPP) all produced contraction of the uterine artery and the umbilical vessels. However, the contraction produced by these agonists was effectively antagonized by the 5-HT_2 receptor antagonist ketanserin, indicating that the contraction was mediated by the 5-HT_2 receptor.

In order to define the relationship between the agonist-induced response and the agonist-receptor interaction, it is extremely important to determine the dissociation constant (K_A value) of the agonist-receptor complex. The reciprocal of K_A is a quantitative measure of the affinity of the agonist for the receptor. As discussed by Furchgott (1966), the same K_A value for a given agonist in different tissue preparations provides strong evidence that the agonist acts on the same type of receptor. In this study, the dissociation constants determined for 5-HT (K_A) and the partial agonists (K_P) are similar among the uterine artery and the umbilical vessels (Table 2), indicating that the agonists are activating the same 5-HT receptors in these tissues. K_A values would also provide very useful information when the relationship between chemical structure and pharmacological activity of a series of agonists acting on a common receptor is being investigated. Once K_A is known, the fraction of receptors ($[RA]/[R_T]$) which must be occupied by the agonist under equilibrium condition to elicit a response of a fixed magnitude can be determined (Furchgott, 1966). The relative efficacy in

Table 2. Dissociation constants (K_A or K_P) of serotonergic agonists and dissociation constants (K_B) of ketanserin vs. the serotonergic agonists in the ovine uterine artery (UA), umbilical artery (UmA) and umbilical vein (UmV)

	K_A or K_P (X 10^{-7}M)			K_B (X 10^{-9}M)		
Agonist	UA	UmA	UmV	UA	UmA	UmV
5-HT	3.70	4.71	4.25	2.00	0.40	2.17
DOM	1.80	0.36	0.85	4.00	0.79	2.78
8-OH-DPAT		49.81	113.89	2.49	0.29	3.47
2-Methyl-5-HT		113.16	89.44	2.88	0.49	1.99
TFMPP		4.34	5.40		1.01	
mCPP		3.15	2.64		0.38	1.45

a series of agonists acting on a common receptor in a given tissue preparation can be obtained by a comparison of the fraction of receptor occupation required to give the same response. In this study, the intrinsic efficacy of DOM is the same as that of 5-HT in the uterine artery, but lower than that of 5-HT in the umbilical vessels. This suggests that DOM is a full agonist in the uterine artery, but is a partial agonist in the umbilical vessels.

In general, antagonists are more selective for receptor subtypes than are agonists. As discussed by Ariens and co-workers (1979), a "complimentarily principle" dictates a sharper differentiation of receptor subtypes by antagonists rather than agonists. Molecules of antagonists usually bear the chemical structure of agonists but are generally larger and more flexible (Ariens et al., 1979). In general, they possess certain lipophilic structural groups and these groups can bind to accessory sites around the agonist binding site of the receptor (Ariens et al., 1979). This may be particularly important in the differentiation of receptor subtypes with variations in accessory sites. For the most part, the definitive classification of the major drug receptor types and subtypes has been accomplished with selective competitive antagonists.

Since competitive antagonists possess no intrinsic efficacy, the potency of a competitive antagonist depends upon its equilibrium dissociation constant (K_B) for the drug receptor. The interaction of a competitive antagonist with a drug receptor is a pure chemical process. The rate of onset and offset of the antagonist with the drug receptor is governed only by molecular forces (Kenakin, 1984). Therefore, the K_B

should be independent of receptor function, location and animal species. No doubt, reliable estimates of K_B values for competitive antagonists are very important in drug receptor classification.

If an antagonist is competitive and the experimental conditions are satisfied, the Schild plot should yield a straight line with the slope being unity. Obviously, if a antagonist is not competitive there will not be a straight line or the slope will be significantly different from unity. On the other hand, the experimental conditions can also affect the Schild plot. Failure to inhibit agonist uptake or metabolism processes in isolated tissues is one of the most common causes of slopes being less than unity (Furchgott, 1972; Furchgott et al., 1973). In order to determine properly the dissociation constant of a competitive antagonist, the sites of loss of the agonist should be blocked. In this study, cocaine was used to block the uptake of 5-HT and iproniazid to block monoamine oxidase (MAO). If a competitive antagonist is not allowed to fully equilibrate with the tissues, the slope of the Schild regression line will be greater than unity. This is more pronounced if drug-receptor interaction and not diffusion is the rate limiting step (Kenakin, 1980). If a given competitive antagonist has a low rate of offset from the receptor, it could behave as an essentially irreversible blocker and produce insurmountable antagonism. This phenomenon has been discussed by Paton and Waud based on the receptor rate theory (Paton and Waud, 1967). The degree of depression of the maximal response is dependent upon the intrinsic efficacy of the agonist (Kenakin, 1984) and the time of incubating the antagonist with the tissue. The interaction of agonist, antagonist and receptor could be in a "hemi-equilibrium"

state as described by Paton and Waud (1967). Under this condition, the equilibrium of the antagonist with the receptor is not changed by the presence of the agonist. The agonist only interacts with a portion of the total receptor population. The depression of the maximum response to 5-HT by methiothepin in this study could be due to the "hemi-equilibrium" state as described by Paton and Waud (1967).

It is apparent that the classification of drug and drug receptor depends on the measurement and comparison of parameters which depend only upon drug and receptor interaction. Obviously, an accurately determined pA_2 or K_B value for a competitive antagonist is extremely important in drug and drug receptor classification. A similar pA_2 or K_B value for a specific competitive antagonist against different agonists provides strong evidence that these agonists act on the same type of receptor. In this study, the dissociation constants of ketanserin vs. 5-HT and the other serotonergic agonists studied are not significantly different in each tissue (Table 2), indicating that they act on the same type of 5-HT receptor, i.e., 5-HT_2 receptor. Since the competitive antagonist possesses no intrinsic efficacy, its dissociation constant should be independent of receptor function, location, and animal species. In the present study, the dissociation constants of ketanserin vs. 5-HT and the other serotonergic agonists determined in the uterine artery and the umbilical vessels are comparable to each other and to those reported in other blood vessels (Cohen et al., 1983; Cohen, 1986; Humphrey, 1984; Van Nueten et al., 1981; Van Nueten et al., 1982). On the other hand, if an agonist-induced response is not antagonized by a specific competitive antagonist, it can be concluded that the respective

receptor is not presented. In this study, the potent and selective 5-HT_3 receptor antagonist MDL 72222 did not antagonize 5-HT-induced vasoconstriction in the uterine artery or in the umbilical vessels, indicating that the 5-HT_3 receptor is not present in these tissues.

SUMMARY

1. The effects of DOM on ovine maternal and fetal cardiovascular functions were investigated in the chronically instrumented ewe/fetus preparation. DOM was administrated intravenously to the ewe. Maternal and fetal heart rates and arterial blood pressures, maternal uterine artery blood flow and fetal umbilical artery blood flow were monitored.
2. DOM produced dose-dependent increases in maternal and fetal blood pressure and a fall in heart rate. There was a linear relationship between the fall in the heart rate and the increase in the mean arterial blood pressure. When the arterial blood pressure increased 1 mmHg, maternal and fetal heart rates decreased 1.4 and 3.4 beats/min, respectively.
3. Maternal uterine artery blood flow was dramatically decreased following DOM administration. A maximum reduction of 92% in the uterine artery blood flow was observed within 5 minutes after DOM injection at 20 μg/kg. The uterine artery blood flow then went into a spasmodic pattern which lasted for more than an hour. The vascular resistance of the uterine artery increased by 6.8, 16.7 and 19.6 fold after DOM injection at 5, 10 and 20 μg/kg, respectively.
4. Fetal intra-abdominal umbilical artery blood flow was also decreased following DOM administration. The maximal reduction in fetal intra-abdominal umbilical artery blood flow was 40% and the spasmodic pattern of blood flow after the

administration of DOM to the ewe was not observed. The maximum rise in the vascular resistance in the fetal umbilical artery was 260%.

5. Maternal arterial blood Po_2, Pco_2 and pH were not affected by DOM in the doses used in this study. Fetal arterial blood pH and Po_2 were decreased 20 minutes after maternal of DOM administration at 10 and 20 μg/kg. Pco_2 increased following DOM administration at 5, 10 and 20 μg/kg, while a reduction in BE was observed at the 20μg/kg dose.

6. Maternal administration of ketanserin (1 mg/kg) produced a fall in maternal arterial blood pressure from 99.7 mmHg to 69.4 mmHg. The blood pressure returned to baseline within 25 minutes. Fetal arterial blood pressure was decreased from 50.9 mmHg to 43.8 mmHg. No significant decrease was observed in maternal and fetal heart rates to ketanserin.

7. Maternal uterine artery blood flow and fetal umbilical artery blood flow decreased after maternal administration of ketanserin, which corresponded to the decrease in the maternal and fetal arterial blood pressures. Vascular resistances of the uterine artery and umbilical artery were not changed by ketanserin.

8. Thirty minutes after ketanserin infusion, maternal and fetal arterial blood pressure responses to DOM were significantly inhibited. Significant increases in maternal and fetal arterial blood pressure were only observed at the higher dose of DOM (40 μg/kg). Maternal and fetal heart rates did not vary

significantly from the baseline at any dose of DOM after ketanserin infusion.

9. The effects of DOM in reducing maternal uterine artery blood flow and fetal umbilical artery blood flow were significantly inhibited by ketanserin. Increases of vascular resistance in the uterine artery and umbilical artery to DOM were significantly inhibited by ketanserin.

10. After ketanserin administration, changes in fetal arterial blood Po_2, Pco_2 and pH became more resistant to DOM.

11. Isolated uterine and umbilical vessels from the ewe and fetus were used to explore in more detail agonist-antagonist receptor mechanisms.

12. 5-HT and DOM potently constricted the maternal uterine artery and fetal umbilical artery and vein and these contractions were effectively antagonized by the selective 5-HT_2 receptor antagonist ketanserin. The dissociation constants (K_B) of ketanserin against 5-HT and DOM were not significantly different in each blood vessel and were comparable among the three different vessels. This indicates that constrictions produced by 5-HT and DOM are mediated by 5-HT_2 receptors in these vessels.

13. DOM was more potent than 5-HT in the uterine and umbilical vessels and possessed a greater affinity for 5-HT_2 receptors in these vessels. In comparison to 5-HT, DOM was a full agonist in the uterine artery but was a partial agonist in the umbilical vasculature.

14. The dissociation constants of 5-HT, DOM and the other serotonergic agonists studied were not significantly different among the three different blood vessels examined.

15. There was little or no 5-HT receptor reserve in the uterine and umbilical vessels.

16. 8-OH-DPAT was a partial agonist in the uterine and umbilical vessels. It is unlikely that contractions produced by 8-OH-DPAT were mediated by 5-HT_{1A} receptor, since the contractions were effectively blocked by ketanserin. The dissociation constant of ketanserin vs. 8-OH-DPAT was similar to that of ketanserin vs. 5-HT in each tissue and was comparable among the three different vessels. This indicates that the contractions produced by 8-OH-DPAT is mediated by 5-HT_2 receptors.

17. 2-MPP, TFMPP and mCPP were partial agonists in the umbilical vessels. However, they lacked (2-MPP), or had weak (TFMPP and mCPP) agonist activities on the uterine artery. These three compounds effectively blocked 5-HT-induced vasoconstriction in the ovine uterine artery. The dissociation constants (K_B) of these compounds as antagonists against 5-HT in the uterine artery were comparable to those K_P values of them as partial agonists in the umbilical vessels.

18. 2-Methyl-5-HT was a partial agonist in the uterine and umbilical vessels. It was unlikely that the constriction produced by 2-methyl-5-HT was mediated by 5-HT_3 receptors. The potent and selective 5-HT_3 receptor antagonist MDL 72222 (10^{-6}M) did not antagonize the vasoconstriction produced by

2-methyl-5-HT. However, the selective 5-HT_2 receptor antagonist ketanserin (10^{-8}M) effectively antagonized the vasoconstriction produced by 2-methyl-5-HT. The dissociation constant of ketanserin vs. 2-methyl-5-HT was similar to that of ketanserin vs. 5-HT in each tissue and was comparable among the different vessels. This indicates that the vasoconstriction produced by 2-methyl-5-HT was mediated by 5-HT_2 receptors.

19. 5-HT-induced vasoconstriction was not antagonized by MDL 72222 (10^{-8}M to 10^{-6}M) in the uterine and umbilical vessels, suggesting that 5-HT_3 receptors were not present in these tissues.

20. Activation of α-adrenergic receptors was not involved in the contraction produced by 5-HT in the uterine and umbilical vessels.

21. The technique of $^{45}Ca^{2+}$ uptake in the smooth muscle cells was employed to explore signal transduction coupling to the 5-HT receptors in the ovine uterine artery. 5-HT and DOM induced concentration-dependent rises in $^{45}Ca^{2+}$ uptake in the uterine artery. The EC_{50} of 5-HT in mediating Ca^{2+} influx was comparable to that which produced vasoconstriction in the same vessel.

22. The Ca^{2+} influx evoked by 5-HT and DOM was antagonized by ketanserin and methiothepin. The dissociation constants (K_B) of ketanserin against 5-HT and DOM regarding their inducing a Ca^{2+} influx were not significantly different from those K_B values in the contraction studies. This indicates that the

Ca^{2+} influx induced by 5-HT and DOM was mediated by 5-HT_2 receptors in the uterine artery.

23. MDL 72222 ($2.5x10^{-6}M$) did not antagonize 5-HT-induced Ca^{2+} influx. This supports the assertion that 5-HT_3 receptors are not present in this tissue.

24. 5-HT-induced Ca^{2+} influx was antagonized by D600 ($2.5x10^{-6}M$) and nifedipine ($2.5x10^{-5}M$) but not by amrinone ($2.5x10^{-5}M$). This suggests that the 5-HT-induced Ca^{2+} influx was mediated by voltage dependent calcium channels.

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